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The Developmental Anatomy of Leaves in *Lolium temulentum*

BY

MARTIN BORRILL

Welsh Plant Breeding Station, Aberystwyth

With four Figures in the Text

ABSTRACT

Measurements of the epidermal cells in *Lolium temulentum* and assessments of cell number indicate that differences in blade width are due mostly to variations in cell number, whereas changes in blade and sheath length result mainly from differences in cell length. A marked increase in length of the flag-leaf sheath, however, was related to an increase in cell number.

Considerable changes in the relative proportions of leaf blade and sheath were observed in the flag leaf, and the leaves immediately preceding it, associated with inflorescence initiation. As one consequence of this the area of the flag leaf, the largest on the shoot, is virtually constant under different environmental conditions. It is suggested that this aspect of correlated development is related to the nutrition of the panicle, since in annual grasses such as the cereals the flag leaf may be responsible for producing up to 30 per cent. of the starch in the grain.

INTRODUCTION

IN a previous study of *Glyceria*, *Lolium*, and *Triticum* species (Borrill, 1959), it was found that the length of successive leaf blades along the main shoot followed a curve which increased to a peak and then fell as the flag leaf was approached. The leaf which was elongating at the time when the inflorescence was initiated produced the longest blade, the succeeding blades being shorter, and these differences in blade length were due largely to differences in the amount of cell extension.

The present paper investigates the contribution of cell size and cell number to the size of the leaf blade and sheath, as affected by inflorescence initiation at the shoot apex, and considers the significance of this pattern in the development of the plant.

A winter-annual inbred-line of *Lolium temulentum*, Ba 6139 was chosen for study. The data recorded include the lengths and widths of successive leaf blades, and lengths of the sheaths, also the stage of inflorescence development on the main shoot. Fifty seedlings were grown in continuous light under greenhouse conditions in boxes of sterilized soil, and top dressings of fertilizer were applied as required to prevent nitrogen starvation. Each leaf was measured when it had stopped growing, which was by the time half of the next leaf was visible.

The shoot apex in three plants was dissected at weekly intervals. Preparations of leaf upper epidermis were made by the lactic acid method developed

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by Clarke (1960). The full width of the leaf blade, was taken from three positions, near the base, at the middle, and near the tip of the leaf. The inner sheath surface was sampled about 1 cm. below the ligule. Photographic prints were made (at a magnification of × 30) of the area half-way between mid-vein and margin to include in the measurements the gradation in cell size, which occurs in this direction. Twenty cells were measured, and the number of cell rows in the width of the blade counted.

EXPERIMENTAL RESULTS

The timing of inflorescence development was controlled experimentally by altering the amount of low-temperature exposure (14 or 28 days) given to the material. A control group of seedlings was grown without low temperature. Before transfer to the refrigerator at between 0 and 5° C. the seeds were moistened with water for 12 hours. The first and fourth leaves were collected from five plants in each treatment and measured. These leaves were chosen to cover the range which occurs during the seedling stage when length of successive leaf blades is increasing.

TABLE 1

Lolium temulentum. *Leaf and Cell Size Data*

Five plants measured in each treatment, and the means used. Macromeasurements in mm. Cell sizes in μ

	Days at low temperature								
	Nil			14			28		
Position of cells on leaf*	Base	Middle	Av.	Base	Middle	Av.	Base	Middle	Av.
Leaf 1									
Leaf length	—	—	139	—	—	111	—	—	109
Cell length	655	609	633	607	580	593	619	627	633
Difference base-middle	46			27			8		
Leaf width	—	—	3·3	—	—	2·6	—	—	3·3
Cell width	313	261	287	225	251	238	264	244	254
Difference base-middle	52			26			20		
No. of cell rows	115	114	114	112	120	116	116	121	118
Leaf 4									
Leaf length	—	—	336	—	—	342	—	—	347
Cell length	982	821	902	1004	947	975	984	891	937
Difference base-middle	161			57			93		
Leaf width	—	—	6·8	—	—	6·6	—	—	7·4
Cell width	363	325	343	315	313	314	409	363	385
Difference base-middle	38			2			46		
No. of cell rows	215	214	214	217	215	216	212	223	217

* Insufficient data were available for the blade tip.

The data are summarized in Table 1, and the results of the analysis of variance are shown in Table 2. The following conclusions may be drawn.

The variance due to genotype is small, demonstrating the homozygosity of this inbred line. The position of the leaf on the shoot is the paramount factor influencing cell size and the gross morphology of the leaf blade and sheath. There is a strong suggestion, significant at 5 per cent., that cell length and cell width are affected by the position of the cells on the leaf. No difference was indicated in the number of cell rows according to position at the base or middle of the leaf blade.

Duration of temperature pre-treatment has an effect on leaf width. This could be accounted for by variation in number of cell rows or in cell width and in both these cases there is a mere suggestion of an effect, significant at from 0·1 to 0·05 per cent.

The way in which low-temperature pre-treatment affects blade width can be assessed in Table 1. There is a reduction in average cell width and blade width in the 14-day treatment. Considering the interactions, first *position of cell on leaf* × temperature, it can be seen that the difference in the cell width between the basal and middle positions on the blade changes according to the duration of low temperature. With nil and 28 days' low temperature the basal cells are wider than the middle cells, whereas in the 14 days' pre-treatment, the basal cells are narrower than the middle cells in leaf 1, but in leaf 4 the basal and middle cells are almost the same length. Secondly, *position of leaf on shoot* × *position on leaf* × temperature, the effect of position on shoot in relation to low temperature again lies in the different response of leaf 1 in the 14-day treatment.

TABLE 2

Analysis of the Variance of Cell Size in Lolium temulentum

		Source of variance				
	Degree of freedom	Cell length	Cell width	No. of cell rows	Blade length	Blade width
Temperature	2	2,114	11,587*	80·0*	341	143·5‡
Genotype	12	7,839	3,499	26·3	563	17·9
Position of leaf on shoot	1	1,522,980§	116,953§	149,600·0§	369,186§	11,213·0§
Position × temperature	2	16,639	7,574†	5·5	1,166†	26·0
Error	12	13,248	1,736	192·1	271	19·6
Position of cell on leaf	1	65,140†	7,063†	224·0	—	—
Position × temperature	2	5,407	4,710†	92·5	—	—
Position × position of leaf on shoot	1	21,403	608	17·0	—	—
Position × temperature × position of leaf on shoot	2	2,331	9,205‡	84·0	—	—
Position × genotypes	12	14,411	910	224·3	—	—
Position × genotypes × position of leaf on shoot	12	8,243	255	109·9	—	—

Levels of significance:

* < 0·1–0·05 ‡ < 0·01
† < 0·05 § < 0·001

It appears that the temperature pre-treatment effect is entirely on average blade width, and this is related to the much narrower cells which occur in the basal region of the first leaf. There is still a suggestion of this effect at leaf 4. Considerable growth of the early leaves takes place in the Petri-dishes at 0–5° C. For instance, leaf 1 may be 30 mm. long after 28 days, but no satisfactory explanation can be advanced to account for the fact that 14 days' low temperature has an effect on cell width and 28 days does not.

The three temperature pre-treatments form part of a single experiment, the main purpose of which is to study the micro- and macro-dimensions of successive leaves. Having confirmed the homozygous nature of the material, a single plant was selected from each treatment and measurements made on all

TABLE 3

Lolium temulentum. *Macromeasurements and Cell Size of Successive Leaves of a Representative Plant in each Treatment*

Cell length and cell width are average figures based on data for the basal and median regions of the blade. Leaf data in mm. Cell data in μ.

Leaf No.	Blade				Cells per leaf	Sheath-length		Blade-length		Blade-width	
	Cell length	Cell width	No. of cell rows in width	No. of cells in length		(Individ.)	(Mean)	(Individ.)	(Mean)	(Individ.)	(Mean)
No treatment											
1	579	25·0	114	250	29,000	33	31	145	142	3·5	3·3
2	—	—	—	—	—	40	43	200	191	4·5	5·1
3	—	—	—	—	—	60	66	260	262	6·0	5·5
4	964	30·8	218	373	84,671	80	77	360	340	7·0	6·8
5	865	30·0	242	450	135,000	110	105	390	364	9·0	8·6
6	920	35·8	264	456	139,992	110	118	420	390	11·0	9·9
7	1,030	34·1	296	393	115,149	160	129	405	386	10·0	9·9
8	895	33·3	302	474	156,420	170	241	425	333	11·0	8·2
9	710	26·0	306	422	178,506	300	361	300	299	11·0	8·0
10	691	22·3	350	405	200,070	490	491	280	287	11·0	8·1
11	660	22·0	384	409	185,686	560	660	270	273	10·0	7·9
Low temperature 14 days											
1	653	26·6	108	188	17,484	25	25	123	116	2·5	2·8
2	—	—	—	—	—	50	46	190	175	5·0	4·4
3	—	—	—	—	—	75	61	280	253	6·0	5·5
4	793	36·6	212	466	96,928	90	80	370	315	7·0	6·7
5	999	27·5	238	395	143,385	110	94	395	371	10·0	8·1
6	1,253	31·6	248	343	108,388	140	117	430	388	10·0	8·7
7	881	37·5	302	497	167,986	162	131	438	397	11·0	9·9
8	725	27·5	268	406	103,124	480	542	295	272	7·0	9·1
Low temperature 28 days											
1	600	20·0	108	200	35,000	23	23	120	101	3·5	3·8
2	—	—	—	—	—	40	42	150	159	6·0	4·5
3	—	—	—	—	—	75	74	230	237	6·0	5·5
4	785	38·3	240	452	94,468	90	103	355	346	8·0	7·1
5	1,300	26·6	256	269	90,922	120	132	350	345	9·0	8·2
6	611	29·2	242	589	140,771	140	139	360	355	7·0	9·1
7	849	36·6	304	412	112,476	150	154	350	368	10·0	9·9
8	675	25·0	364	592	284,160	480	464	400	276	12·0	9·8

the leaves of the main shoot. Data for leaf length, width and sheath length were also obtained for all the 50 genotypes in each treatment. The results are given in Table 3, and Figs. 1 and 4 illustrate the trend in the morphology of successive leaves.

The length of successive leaf blades increases to a maximum at the time of inflorescence initiation and then decreases, except in the case of the 28 days' pre-treatment, where this relation is disturbed and the inflorescence is initiated at leaf 2.

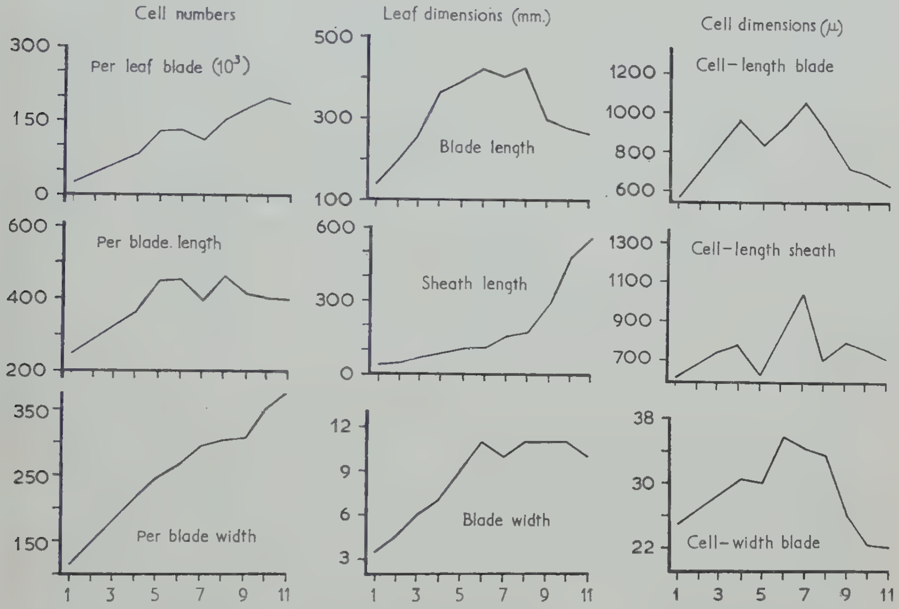

FIG. 1. Cell numbers, and cell and leaf dimensions in *Lolium temulentum* leaf sequences of different ages (1–11, horizontal scales), grown without low-temperature pre-treatment. Similar curves were obtained with 14 or 28 days' low-temperature.

It was previously reported (Borrill 1959) that when a spring annual line of *L. temulentum* was grown under continuous light, the inflorescence was initiated precociously, at the second leaf. It seems likely, therefore, that low temperature and supplementary light are replaceable as 'unnatural environments' in which the correspondence of inflorescence initiation with the elongation of the longest leaf blade is disturbed.

As the duration of low-temperature is increased the inflorescence is initiated progressively earlier. There is, however, no corresponding reduction in leaf number between the 14 and 28 days' treatments (Fig. 4), and the size and pattern of successive leaves is little influenced by giving 28 days' low temperature instead of 14.

The curves for cell size are based on the means for the basal and median parts of the blade. The data available for the blade tip were incomplete. The omission of the tip data did not make a significant or consistent difference in the means, and, bearing in mind the small effect of position on leaf on cell length shown in the analysis, the assessment of cell length used here seems reliable.

There is a significant regression of blade length on cell length, Fig. 2, borne out by the curves in Fig. 1, although in the 28-day pre-treatment the curve for leaf length departs somewhat from that obtained for cell length.

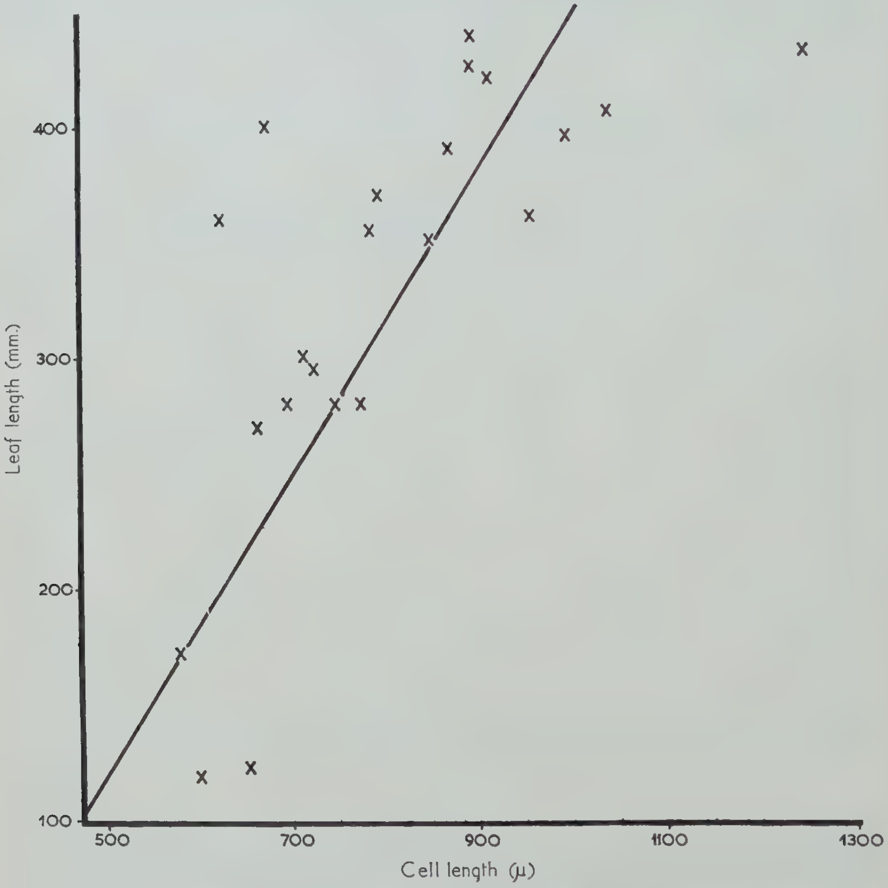

Fig. 2. The regression of leaf length (y) on cell length (x) in *Lolium temulentum*. Data from three temperature pre-treatments. $y = 1.55x + 325$.

In each treatment successive leaf sheaths show a regular increase in length, culminating in a spectacular increase at, or just before, the flag leaf stage. In the control, and 14-day treatments, this increase occurs immediately following inflorescence initiation. The resultant curve for the whole leaf (blade and sheath) is shown in Fig. 4, using the mean figures for 50 plants, the trend of increasing length almost ceases at the 4- to 6-leaf stage, finally increasing sharply to the flag leaf.

In this experiment the leaf area has been assessed as the product of leaf length and blade width. The results slightly exaggerate the area of the blade (regarded as rectangular) relative to the sheath, which is wider than the blade. Trial data indicate that variation in blade and sheath width are in proportion so that this bias does not vary greatly from one leaf to another. In the control a decline in leaf area after leaf 4 is reversed following inflorescence initiation, and with 14 or more days of low temperature leaf area rises continually. In each environment the area of the flag leaf is nearly the same.

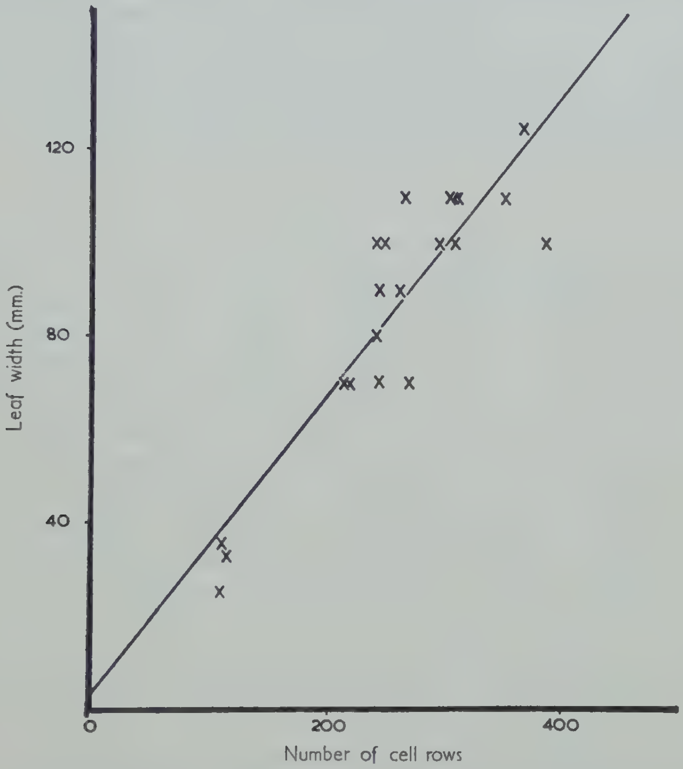

FIG. 3. The regression of leaf width (y) on number of cell rows (x) in *Lolium temulentum.* Data from three temperature pre-treatments. $y = 3.22x + 3{\cdot}5$.

The cell-length curves in Fig. 1, although rather irregular, indicate that cell length and sheath length tend to increase together, but the final big increase in sheath length is associated with a sharp reduction in cell length, which suggests that extensive cell division has taken place in these leaves, the last to be formed.

In each treatment, blade width, cell width (average of base and middle), and number of cell rows increase together up to the stage of leaf four. The analysis, Table 2, indicated that position on the shoot affected cell width, and number of cell rows, up to leaf 4. Subsequently the curves for blade width, and number of cell rows follow one another very closely, and a significant overall regression is found of blade width on number of cell rows (Fig. 3).

The curve for cell width tended to be erratic after the fourth-leaf stage, and no significant relation was found with leaf width.

The number of cells in the length of the leaf blade was estimated by dividing the blade length by the mean cell length. The resulting curves are irregular for each treatment, but taken overall show a trend in relation to blade length although there was no significant regression of blade length on cell number. It is clear that in this material cell length is the main factor affecting blade length, although there is a good deal of variation in the number of cells present.

The number of cells in each leaf blade was arrived at by multiplying the mean number of cell rows by the number of cells in the length of the blade. Since the number of cell rows shows a regular increase, the resulting curves parallel those obtained for number of cells in the blade length.

DISCUSSION

There have been few recent studies in the *Gramineae* concerning the relative contribution of cell size and number to leaf size. Soper and Mitchell (1956), measured the size of cells from three positions on the lower epidermis of the second and sixth leaves in *Lolium perenne*. They counted the number of cell rows across the base of the leaf, and estimated the number of cells in the length of the blade. Because of the heterozygosity and consequent high between-plant variance of perennial ryegrass no significant differences in cell length or width could be detected between the second and sixth leaves, or in cell size along the blade. The number of cell rows in the blade width increased from 134 in leaf two, to 188 in leaf six. Similar differences were found in number of cells in the length of the leaf, as would be expected if these figures were obtained by dividing leaf length by mean cell length.

These data also show that increases in blade width are associated with increases in number of cell rows, as is found here in *Lolium temulentum*, but they do not reliably indicate the relative contribution of cell length and cell number to blade length, or of cell width to blade width. The high variability of outbreeding grasses raises difficulties in this type of study.

Stuckey (1942) investigated leaf morphology in *Dactylis glomerata* grown in long and short days, collecting the youngest leaf from several plants every 14 days, which very approximately represented successive leaves on the shoots. Leaf length and number of epidermal cells per unit area were measured. It is not known whether upper or lower epidermis was used. The length of successive blades was found to increase, while the number of cells per unit area decreased (the leaves were always larger in long days), hence the longer leaves had larger cells. The number of cells in the length of the leaf also increased.

The author concluded that 'lengths of vessel elements, of fibres, and of the epidermal cells varied directly with organ size, indicating that leaf length differences were due to cell size and not to cell number, at least with respect to these cell types'. The increase in number of cells in the length of the leaf was not emphasized, although this indicates some effect of cell number as well.

Zalensky (1904) according to Ashby (1948a), 'found that the epidermal cells in successive leaves of *Dactylis* decreased from node to node of the stem. It is clear from Zalensky's data that there is a negative correlation between cell size and leaf size in the first seven leaves of *Dactylis glomerata*, and that a change in shape measured as the ratio of $\frac{\text{length}}{\text{breadth}}$ accompanies decreasing cell size.' The situation reported by Zalensky is the opposite of that observed by Stuckey, and data are being obtained by the writer to elucidate the point.

All the above instances suggest that there is a relation between increasing blade length and cell size, and indicate that there may be an effect of cell number as well. The data given below, taken from Table 1, suggest the relative importance of cell size and number in relation to blade width.

		Treatment (days of low temperature)					
		0		14		28	
	Leaf No.	No. of cell rows	Blade width	No. of cell rows	Blade width	No. of cell rows	Blade width
No. of cell rows	1	114	3·3	116	2·6	118	3·3
	4	214	6·8	216	6·6	217	7·4
Relative increase		1·9	—	1·9	—	1·9	—
Cell width	1	287	—	238	—	254	—
	4	343	—	314	—	385	—
Relative increase		1·2	—	1·3	—	1·5	—

From leaves one to four, the relative increase in number of cell rows (1·9) is greater than the increase in cell width (1·2 to 1·5). This is borne out by the trend in the later leaves, the curves for cell rows and leaf width follow one another very closely, whilst cell width is erratic (Figs. 1–3). There is a significant regression of width on cell number, not on cell width. It seems reasonable to suppose that number of cell rows is the main factor affecting blade width, and this may be related directly to the size of the subapical region of the shoot.

The leaf as a whole passes through successive stages of cell division, elongation and maturation, as described by Prat (1948). The data of Soper, of Stuckey, and of the author indicate that the length of successive blades is largely related to amount of cell elongation, as indicated by the regressions obtained for *Lolium temulentum* and *Triticum.*

The fact that leaf blade length and blade width depend on different anatomical factors suggests that they may be inherited independently.

The present data merely indicate that sheath length is related to cell length. It might be expected that the length of sheath and blade would depend on the same factor, since developmentally and morphologically they are very similar, the ligule being formed later as a superficial outgrowth. However, the data show that the sheath differs from the blade in that the final rapid increase in sheath length at the flag leaf is due mainly to increase in cell number.

In many dicotyledonous plants successive leaves on a shoot show a trend of xeromorphism, in that the leaves are progressively smaller, with some reduction in cell size, as in *Ipomoea* (Ashby, 1948*b*). The author's data, and those of other investigators, with the exception of Zalensky, indicate that herbage grasses do not show this trend in the vegetative phase of growth, but that successive blades and sheaths are larger with bigger cells. After inflorescence initiation the blades may, or may not, become progressively xeromorphic, depending on previous environmental circumstances. The sheaths continue to increase in length, and the length of the whole leaf, blade and sheath, in general also increases along the shoot.

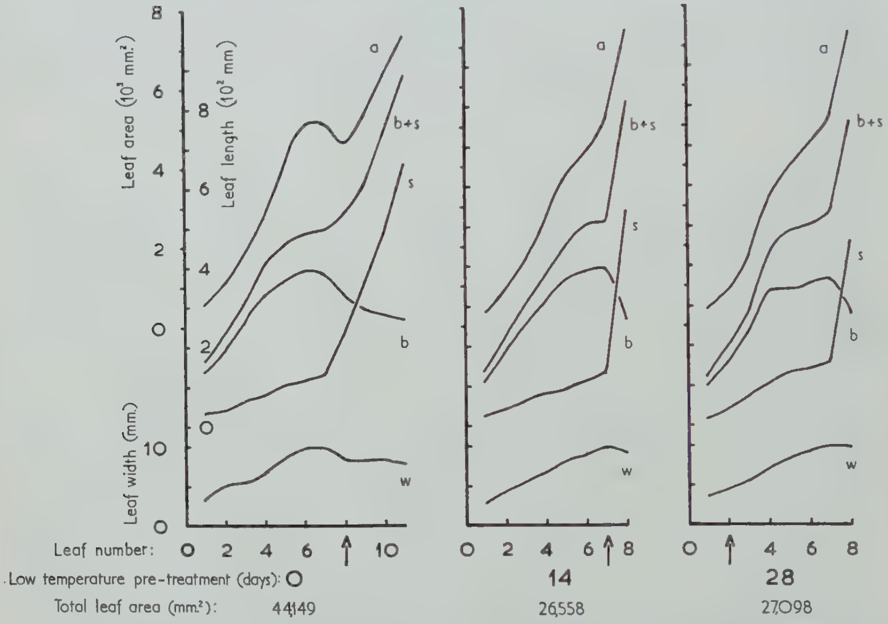

FIG. 4. Size of successive leaves on the main shoot in *Lolium temulentum*, means of 50 plants per treatment.
a. leaf area *b*. blade length *s*. sheath length
b+*s*. total leaf length *w*. leaf blade width
↑ leaf stage of inflorescence initiation.

The effect of increasing the amount of low-temperature pre-treatment is to alter the timing of inflorescence initiation, the number of leaves found on the shoot, and to bring about the spectacular change in the relative proportions of blade and sheath which occur when passing up the shoot from the leaf with the longest blade to the flag leaf. As the amount of low-temperature pre-treatment increases the total leaf area tends to fall; nevertheless, the area of the flag leaf remains virtually constant. Fig. 4 summarizes these changes in *Lolium temulentum*.

The grass shoot exhibits a complex developmental pattern into which inflorescence initiation, leaf morphology, ear production, and nutrition are integrated. Several studies have been made of the relative importance of leaf blade and sheath, culm, and floral parts in providing material for the formation of the panicle and starch in the ear of wheat and barley. These results indicate that each tiller is nutritionally autonomous (Archbold, 1945), and that the leaf blades, sheaths, and the culm provide between 55 per cent. (Porter, Pal, and Martin, 1950) and 60 per cent. (Archbold, 1945; Boonstra, 1936) of the material in the ear, 30 per cent. of which, according to the data of Porter *et al.* is contributed by the flag-leaf blade and sheath.

In the flag leaf the blade is small relative to the sheath and photosynthesis in the latter is therefore important, this leaf is also the last to turn yellow and hence the last to stop photosynthesizing.

It is possible that these findings can be applied to *L. temulentum* which has been selected for a grain size similar to that of the cereal crops in which it occurs as a weed. In this case we can relate the pattern of leaf size along the shoot in the cereals and *L. temulentum* in part to the nutritional requirements of the ear. The fact that flag-leaf area remains relatively constant in *L. temulentum* under different environmental treatments supports this view.

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Some Aspects of Energy Flow in Plantations of *Pinus sylvestris* L.

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ABSTRACT

Calorific values are given for the different types of plant material in an age series of plantations of *Pinus sylvestris* and the amounts of energy in the organic matter present per hectare of woodland are calculated. Following afforestation with Scots pine a progressive accumulation of energy occurs up to 35 years of age when the organic matter present per hectare contains about 10,000 × 10^8 calories. Values are given for the energy content of the primary production by the trees and ground vegetation, of energy accumulation in the tree stock, understory vegetation, and litter, of energy removed in the harvested trees and of energy released by decomposition. The photosynthetic efficiency of the forest community is calculated and shown to be comparable to that of high-producing agricultural crops so that it is suggested that the forest community makes very full use of site conditions.

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INTRODUCTION

USING data, mainly for aquatic communities, Lindeman (1942) pointed out that living organisms can be grouped into a series of trophic levels with each level depending upon the preceding one for its energy supply so that ultimately the energy requirements of the community have to be met by solar radiation trapped by the green plants and converted to chemical energy in the plant material.

In woodland communities the photosynthetic efficiency of the trees and understory vegetation sets a maximum limit to the metabolism of the ecosystem. A knowledge of the overall rates of energy fixation, release, and accumulation by woodland plants is needed to provide a basis for integrating the diverse processes concerned in energy flow at all trophic levels in woodland communities. Only a small proportion of the incident solar radiation

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is utilized in photosynthesis since most of the incoming energy is expended in various ways, e.g. by evaporation and transpiration, by reflection, and as net long-wave loss to the atmosphere.

In certain forests, such as old natural woodlands which are relatively stable, productivity and decomposition may be equal so that the amount of organic matter in the ecosystem remains constant from year to year and energy flow is such that energy input and output balance. Instances of equilibrium in woodlands appear to occur only rarely under present-day conditions and usually the amount of organic material and hence of energy retained within woodland communities is increasing or diminishing.

If the vegetation of the British Isles was allowed to develop without human interference, forests would probably become established over most of the land area and in fact afforestation is happening artificially since considerable areas are now being planted with trees. Because few natural woodlands remain, studies of their energy flow have not been made in this country, but it is of interest to examine the progressive changes that are taking place over the first rotation of the planted woodlands. Whittaker (1953) has suggested that succession towards the climax is normally characterized by increasing complexity, diversity, productivity, and stability of the community. At Thetford Chase, in England, plantations of *Pinus sylvestris* which form an age sequence from recently planted heathland to pine forest have been sampled intensively in the course of investigations of woodland productivity and nutrient turnover (Ovington, 1957, 1959*a*, 1959*b*). Determinations of the energy content of the collected plant samples have been made and are presented in this account to illustrate the broad features of the changing pattern of energy utilization and flow as woodland conditions develop.

METHODS

The method of sampling the pine plantations was described in a previous paper (Ovington, 1957). The calorific value of the collected plant material was determined by igniting compressed pellets of dry, finely ground organic matter in an oxygen bomb calorimeter and making the necessary corrections to compensate for the free acids released in combustion. The heat unit used throughout this account is the gram-calorie, that is the amount of heat required to raise the temperature of 1 gramme of water 1 degree centigrade. The energy contained in the plant material present over a hectare of land was calculated by multiplying the calorific value by the dry weight of organic matter per hectare.

RESULTS

1. *Calorific values of different types of organic matter*

The heat of combustion values for the different types of plant material collected in the pine plantations ranges from 2,859 to 5,223 calories per gramme of oven-dry matter (Table 1). Tree leaves, particularly the older leaves, give the greatest calorific values which, for the living trees, decrease in the order leaves, branches, cones, and roots. The small values for tree rootlets result partly from soil particles adhering to them since it is difficult to ensure that all mineral soil is removed from small rootlets during washing. The heat of combustion figures for the ground vegetation are relatively constant despite the fact that the understory vegetations vary greatly in species composition and have grown under very different light conditions because of differences in the density of the tree cover.

TABLE 1

Calorific Values of Plant Material as calories per gram

Tree age in years	7	11	14	17	20	23	31	35	55	Average
Trees										
Bole	4,910	4,792	4,778	4,804	4,779	4,841	4,638	4,802	4,684	4,781
Living branches	4,939	4,882	4,962	4,932	4,884	4,880	4,869	4,744	4,939	4,892
Dead branches	—	4,957	4,986	4,965	4,948	4,865	4,777	4,908	5,086	4,937
Cones	—	—	—	4,699	4,757	4,649	4,723	4,780	4,692	4,717
Leaves 1 year old	5,000	5,041	4,986	5,025	5,071	4,894	5,042	5,029	4,973	5,007
Leaves 2 years old	4,989	5,164	5,002	5,113	5,149	5,034	5,085	5,179	5,122	5,093
Leaves 3 years old	5,135	5,194	5,116	5,207	5,177	5,137	5,152	5,214	5,083	5,157
Roots > 0·5 cm. diameter	4,684	4,746	4,800	4,746	4,659	4,556	4,894	4,937	5,040	4,785
Rootlets < 0·5 cm. diameter	—	—	3,949	4,048	4,126	3,438	2,642	3,094	3,768	3,581
Ground flora	4,840	4,242	4,434	4,856	4,863	4,678	4,492	4,635	4,469	4,612
Humus layers										
Fresh litter—branches	—	—	—	—	4,992	4,907	4,882	4,975	4,890	4,929
Fresh litter—cones	—	4,669	4,788	4,782	4,790	4,851	4,802	4,831	4,786	4,787
Fresh litter—leaves	5,147	4,986	5,084	5,114	5,165	5,119	5,223	5,162	5,108	5,123
Fresh litter—ground flora	4,846	4,512	4,092	4,881	4,867	4,581	4,594	4,510	4,635	4,613
F layer	—	4,388	4,681	5,086	5,131	4,890	4,351	4,917	4,489	4,742
H layer	3,151	3,212	3,499	4,478	4,219	3,369	3,613	3,732	2,859	3,570

The fresh surface litter was separated out according to whether it originated from tree leaves, branches, cones, or the ground vegetation and the differences in energy content of the litter components broadly parallel the differences recorded for these when they are parts of living plants. There is a slight decrease in the calorific value of the humus layers with increased decomposition, but the large decrease in heat of combustion values from the F to the H layer results partly from the mixing of mineral soil into the lowermost humus.

2. *Energy relationships on an area basis*

The trees and ground vegetation are the primary producers of organic matter in the pine plantations and to some extent are complementary in that the ground vegetation is most productive in the early and late stages of plantation development when the tree cover is not so dense and the trees are less productive. Over the 55-year period the total dry-matter production by the trees and ground flora amounts to 682,000 kilograms per hectare, which corresponds to about $42{,}154 \times 10^8$ calories and gives an average annual fixation of 766×10^8 calories. After 55 years the trees are responsible for about 78 per cent. of the energy fixed by the woodland community.

The seven-year-old trees are small, and it is unlikely that they have caused any great changes in the ground vegetation and humus layers, so we may assume that the amounts of energy in the ground vegetation and litter of the youngest plantation correspond fairly closely to those present before the trees were planted. Following afforestation with Scots pine, there has been a progressive accumulation of organic matter and hence of energy present per hectare of woodland from 1,110 × 10^8 calories initially to 10,019 × 10^8 calories at 35 years of age (Table 2). From 35 to 55 years there is a decrease in the energy stored in the organic matter of the ecosystem to 9,125 × 10^8 calories per hectare, this occurs primarily as a result of the foresters harvesting many large trees during this period. Over the whole 55 years the average annual increase in the amount of energy held in the ecosystem is about 146 × 10^8 calories per hectare. Much of the stored energy is contained in the living trees in which it has accumulated at an average rate of 138 × 10^8 calories per hectare per annum and in the older plantations this energy is present mainly in the tree trunks and roots. The bulk of the remaining energy is in the humus layers because the ground flora is not very luxuriant beneath the dense forest canopy and the forest understory vegetation does not contain as much energy as the vegetation of the unplanted heath.

TABLE 2

Energy Content of Pine Plantations as 10^8 gram calories per Hectare

Tree ages in years	7	11	14	17	20	23	31	35	55
Trees									
In bole production	50	252	402	780	1,296	2,875	5,919	7,860	12,454
In living boles	50	252	402	780	1,296	2,147	3,787	4,745	4,529
In boles of harvested trees	0	0	0	0	0	728	2,132	3,115	7,925
In branch production	50	262	470	1,024	1,365	2,835	3,831	4,633	5,126
In living branches	47	210	387	465	586	675	458	455	605
In dead branches on tree	0	2	70	126	323	634	642	749	510
In branch litter fall	3	50	13	433	456	1,526	2,731	3,429	4,011
In cone production	0	0	5	61	229	368	481	576	1,209
In living cones	0	0	2	34	82	19	10	34	23
In cone litter fall	0	0	3	27	147	349	471	542	1,186
In leaf production	134	496	1,089	1,742	2,297	2,824	4,290	5,059	9,523
In living leaves	106	297	335	456	537	251	420	505	365
In leaf litter fall	28	199	754	1,286	1,760	2,573	3,870	4,554	9,158
In root production	125	415	443	565	627	1,578	2,090	3,304	4,582
In living roots	125	415	443	565	627	1,184	1,175	2,015	1,557
In roots of thinned trees	0	0	0	0	0	394	915	1,289	3,025
In total tree production	359	1,425	2,409	4,172	5,814	10,480	16,611	21,432	32,894
In living trees (including dead branches)	328	1,176	1,639	2,426	3,451	4,910	6,492	8,503	7,589
In boles and roots of thinned trees	0	0	0	0	0	1,122	3,047	4,404	10,950
In total litter fall from trees	31	249	770	1,746	2,363	4,448	7,072	8,525	14,355
Ground flora									
In ground flora production	3,927	5,677	6,297	6,468	6,510	6,540	6,848	7,170	9,260
In living ground flora	561	314	99	15	13	7	70	91	118
In litter falling from ground flora	3,927	5,924	6,759	7,014	7,058	7,094	7,339	7,640	9,703
Litter layers									
In litter fall from trees and ground flora	3,958	6,173	7,529	8,760	9,421	1,542	14,411	16,165	24,058
In existing litter layers	549	431	396	811	1,158	1,091	1,191	1,425	1,418
Released by decomposition	3,958	6,291	7,682	8,498	8,812	11,000	13,769	15,289	23,189
Accumulated in tree stock, ground flora, and litter	1,438	1,921	2,134	3,252	4,622	6,008	7,753	10,019	9,125

The accumulation of energy that is associated with the build-up of organic matter as forest conditions develop represents only a small part of the energy fixed by photosynthesis, since energy is lost from forest ecosystems by plant respiration and other metabolic processes, by the harvesting of tree stems, and by the breakdown of plant material under various plant and animal agencies (Table 3). No measurements of respiration have been made in the pine stands but it is possible to estimate the loss of energy through harvesting and the breakdown of plant material.

In the pine plantations harvesting of the trees did not begin until after the twentieth year but from then onwards the average annual loss of energy per hectare through the removal of tree boles is 226×10^8 calories and for the whole 55-year period is 144×10^8. The tree crowns and roots are not harvested but are left to decompose in the forest stands. The crowns of the felled trees are scattered as litter over the forest floor where decomposition occurs fairly rapidly but the roots are left in the soil and large roots normally persist for many years. If the roots of the harvested trees decomposed completely within the 55-year period the average annual release of energy would be 55×10^8 calories per hectare. It seems possible that some roots of the harvested trees may survive and become part of the root systems of the remaining trees since natural fusions between roots of different trees were frequently observed when the root systems were excavated. Unfortunately we have no quantitative data of root survival and decay in the pine stands.

TABLE 3

Energy Budget for Pine Plantations (10^8 *gram calories per Hectare*)

Tree age in years . . .	7	11	14	17	20	23	31	35	55
Change in energy content of ecosystem (tree stock, ground flora, litter) . . .	+328	+811	+1,024	+2,142	+3,512	+4,898	+6,643	+8,909	+8,015
Energy in roots of harvested trees	0	0	0	0	0	394	915	1,289	3,025
Energy removed in boles of harvested trees. . . .	0	0	0	0	0	728	2,132	3,115	7,925
Energy released by decomposition	3,958	6,291	7,682	8,498	8,812	11,000	13,769	15,289	23,189
Energy content of primary production by trees and ground flora	4,286	7,102	8,706	10,640	12,324	17,020	23,459	28,602	42,154

The breakdown of plant material occurs by diverse processes: in some cases living plants may be eaten by animals and in others dead plant material may fall as litter from the trees and ground flora on to the forest floor where decomposition occurs. These processes have not been evaluated separately in this investigation but have been grouped together as litter breakdown because ultimately all result in the complete oxidation of the plant material. Apparently litter fall and decomposition on the forest floor are of the greatest importance in the pine plantations. Although the weight of litter over the mineral soil of the pine plantations increases as the forest stands mature, the increase is small compared to the amount of leaf, branch, and cone material that is decomposed. After 55 years the total litter fall per hectare contains $24{,}058 \times 10^8$ calories but the corresponding increase in energy content of the litter layers is only 869×10^8 calories so that $23{,}189 \times 10^8$ calories have been released as the organic matter is oxidized. Over the 55-year period covered by the pine plantations the average annual release of energy by the breakdown of plant material other than tree roots is 421×10^8 calories per hectare.

True woodland conditions occur from 17 to 35 years of age when the tree crowns form a fairly continuous and deep cover and forest productivity is greatest so that it is of interest to record the energy relationships over this period. Between 17 and 35 years the trees are responsible for most of the primary production, which on average amounts annually to 998×10^8 calories per hectare, of this 376×10^8 calories are retained within the ecosystem, 173×10^8 calories are removed in the harvested tree stems, 72×10^8 calories are left in the roots of the harvested trees, and 377×10^8 calories are released by decomposition.

3. *Efficiency of energy conversion*

Knowing the annual amount of solar radiation reaching the vegetation surface and the amount of energy in the primary production of a plant community, the over-all efficiency of solar energy utilization for the production of organic matter can be calculated if respiratory activity is excluded. Whilst such calculations of photosynthetic efficiency may be subject to considerable error it is interesting to consider the forest plantations from this view-point because from dry-weight production data forests appear to be one of the most productive forms of land use in the Thetford Chase region.

Unfortunately no direct measurements of incident solar radiation at Thetford Chase are available and since different methods of determining incident radiation are employed it is important to describe precisely the method used. Blackman and Black (1959) and Hellmers and Bonner (1960) have made preliminary estimates of energy utilization from the dry-weight data of the pine stands published previously (Ovington, 1957) and from the solar radiation records for Kew. Blackman and Black using records collected at Kew from 1947 to 1957 give an annual mean value for radiation received on a horizontal surface of $96{,}000\times10^8$ calories per hectare whilst the corresponding figure for Hellmers and Bonner based on tables published by Gorczynski (1954) for the period 1913 to 1920 is $75{,}000\times10^8$ calories per hectare. The figure given by Blackman and Black is too high due to an unfortunate error in the calculations and the annual mean should be about $81{,}000\times10^8$ calories per hectare (Penman, personal communication). Only part of the incident energy is photosynthetically active and if the incident radiation is restricted to the effective photosynthetic wavelength range of 4,000 to 7,000 Å the annual figure quoted by Blackman and Black becomes $42{,}000\times10^8$ calories per hectare and that of Hellmers and Bonner $39{,}000\times10^8$ calories per hectare.

The pine plantations at Thetford Chase are situated about 150 kilometres north-east of Kew and consequently may not receive the same amount of annual radiation as at Kew. An empirical local estimate of average annual radiation at Thetford Chase can be made from monthly weather data collected at Mildenhall climatological station close to the pine plantations using an equation known to be fairly reliable over south-east England (Penman, 1948). This equation gives a value of $76{,}700\times10^8$ calories per hectare for the mean annual income of short-wave radiation (Rc). From the same data the mean annual net long-wave radiation loss (Rb) is equivalent to about $32{,}000\times10^8$

calories per hectare. Assuming a reflection coefficient (r) of 0·25, which applies to agricultural crops (Monteith 1959) and is probably too large for pine forests, the net radiation (H) can be estimated using the formula $H = Rc(1-r)-Rb$ and is $25{,}500 \times 10^8$ calories per hectare. This value is equivalent to the energy required to evaporate about 43 cm. of precipitation per annum which is very close to the known annual evaporation so that the quantity of energy used in photosynthesis is likely to be relatively small.

Blackman and Black and Hellmers and Bonner converted the dry-weight values for the organic matter of the plantations to energy values by assuming conversion factors of 4,500 and 4,000 calories per gramme of organic matter respectively. The calorific values of the plant material vary considerably and the average value obtained by use of the bomb calorimeter is 4,700 calories per gramme (Table 1). In evaluating the efficiency of energy conversion by forest ecosystems the energy content of each type of organic material should be calculated separately according to its true calorific value and it is important to distinguish between the energy contained in the primary production of organic matter and the change in the energy held within the ecosystem as a result of changes in the biomass.

TABLE 4

Average Percentage Utilization of Total Incident Solar Radiation

Tree age in years	17–35	0–55
Contained in primary plant production	1·30	1·00
Accumulated in living trees	0·44	0·18
Accumulated or lost from ground flora	$+<$ 0·01	$-<$ 0·01
Accumulated in litter layers	0·04	0·02
Contained in boles of harvested trees	0·23	0·18
Contained in roots of harvested trees	0·09	0·07
Released by plant decomposition	0·49	0·55

If the value of $76{,}700 \times 10^8$ calories per hectare is accepted for the annual income of solar radiation to the pine plantations, then on the average about 1 per cent. of this energy is contained in the primary production of organic matter by photosynthesis over the 55 years and 1·3 per cent. during the most productive period (Table 4). The average percentage of energy retained in the ecosystem is much less being only 0·2 per cent. during the 55 years and 0·5 per cent. from 17 to 35 years. If the incident radiation figures are limited to the photosynthetic range of 4,000 to 7,000 Å the percentage utilization values would be approximately doubled and if the net radiation figure, calculated by the Penman formula and with an allowance for light reflection, is used as a measure of incident radiation the percentage utilization value would be almost quadrupled (Ovington and Heitkamp, 1960).

DISCUSSION

The Thetford heaths and the pine plantations are both unnatural representing different types of land utilization but the forest cover probably approximates most closely to the type of vegetation that would develop if the area was left without human interference. The recent change in land use with afforestation has greatly affected the community dynamics and in particular there has been a general increase in the rate of energy fixation, flow, and release; since energy fixation exceeds the other two processes energy has accumulated in the forest ecosystems. The higher rate of energy fixation by the woodlands may be attributed to the large leaf and root mass developed by the trees which enables them to make very full use of the site.

In these investigations no account has been taken of the energy content of the fauna but obviously the establishment of the trees will also affect the species composition and biomass of the fauna. Lack (1939) has described the changes that occur in the species composition of the Breckland avifauna following afforestation but the change in the energy stored in the animals is likely to be much less than that in the plants (Gibbs, 1960) since the biomass of the fauna is relatively small and energy flows more rapidly through the animal bodies.

Comparisons of the photosynthetic efficiencies of different types of plant community have been confused by failure to distinguish between values with and without a correction for respiration and values based on the total incident light energy and the energy available for photosynthesis. Wassink (1959) has reviewed much of the available data of photosynthetic efficiency and concludes that, when energy fixation is measured as the energy content of the organic matter produced and the incident radiation figures are limited to wavelengths available for photosynthesis, the overall annual photosynthetic efficiency for field crops in Holland lies between 1 and 2 per cent. although over short periods of a few weeks efficiencies of 7 to 9 per cent. may be obtained. The corresponding overall annual efficiency for the pine plantations is about 2·5 per cent. so that pine plantations represent an efficient photosynthetic system presumably due in part to the longer growing period. Scots pine is not the most productive forest type of the region and plantations of Corsican pine and Douglas Fir nearby give dry-matter increments two and three times that of the Scots pine.

In general there has been a tendency to regard forestry as a rather unproductive form of land use but it is evident that forest plantations properly managed are capable of making very efficient use of a site. It is not clear whether growth is being limited by nutrients, water, or radiation, but the forest plantations have a high leaf-area index and it is noteworthy that during the period of fastest tree growth light on the forest floor is greatly reduced and the understory vegetation is absent. Evidently at this stage the biological productivity of the forest may be close to the maximum site potential, and this is a factor of considerable interest to field biologists in considering possible forms of land use.

ACKNOWLEDGEMENTS

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Abnormalities of the Reproductive System of *Pinus contorta* Loudon*

BY

T. M. BLACK

With four Figures in the Text

ABSTRACT

Various abnormalities and their occurrence within individual trees are described. It is noted that the abnormalities, e.g. replacement of male by female strobili, are usually found on shoots situated in the upper crowns of trees. The occurrence of bisexual strobili, and the proportion of female tissue in them, is also apparently influenced by the proximity of normal female cones. Zobel's (1952) conclusion that abnormalities are often associated with a physiological upset caused by a shock, e.g. planting out of the species' natural range, is discussed. It is concluded that the prevalence of abnormalities in trees of inland origin, and their absence from individuals of the coastal type, is associated with this.

INTRODUCTION

THE observations on the abnormalities described were made during a study of the morphological characters of twelve provenances of *Pinus contorta* Loudon. Seven of the provenances were of inland origin, and the other five coastal. (Table I). The trees examined were growing in a provenance trial situated at Millbuie Forest, on the Black Isle, Easter Ross.

DESCRIPTION OF ABNORMALTIES

Abnormal Production of Female Cones

In *Pinus contorta* Loudon the cones are normally produced either sub-terminally or laterally at intermediate nodes on the shoot, and may be present singly or in groups of up to five. Both sub-terminal and lateral cones may be present on the same shoot. In contrast, the abnormally produced cones occur as follows:

1. The cones arise in large clusters at the base of the shoot, and give the impression of having replaced the male inflorescence (Fig. 1).
2. (*a*) The cones arise in a small cluster, ten being the largest number recorded, near the base of the year's shoot growth but separated from the base by numerous fascicles of needles.
    (*b*) The cones arise in a small cluster, never more than six have been observed, well above the base of the year's shoot growth.
    (*c*) The cones arise in small numbers, interspersed with needles, near the base of the shoot (Fig. 2).

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* This article is based on part of a thesis submitted for the degree of Ph.D. at the University of Aberdeen.

[Annals of Botany, N.S. Vol. 25, No. 97, 1961.]

3. In rare cases female cones have arisen in the midst of a male inflorescence.

In the specimens of type 1 which were examined the mature cones, although they produced apparently fertile seeds, were only half the size of the normal female cones produced by the tree. Moreover, while the normal cones examined were invariably reflexed the abnormal ones generally had their axes at an acute angle to the shoot.

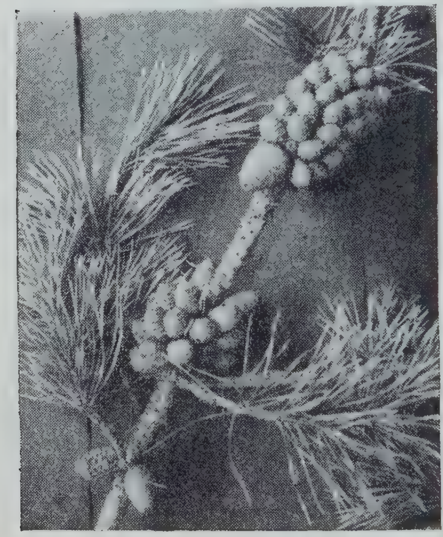

FIG. 1. Clusters of cones situated at the bases of the shoots.

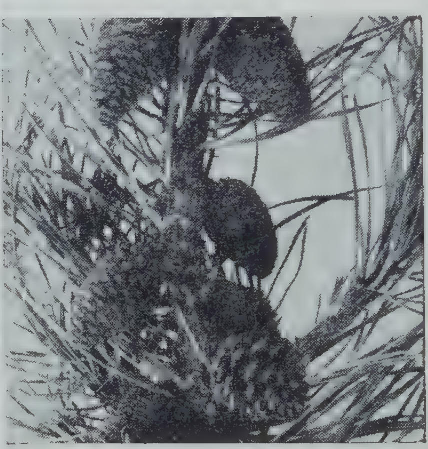

FIG. 2. Mature cones interspersed with needles at the base of a shoot.

In types 2(*a*) and 2(*b*) only current year cones were available for examination. However, in size, colour, and structure they were apparently normal for the trees which bore them, only their position being abnormal. One specimen of type 2(*c*) with mature cones was found and, in this case, the abnormally produced cones were only slightly smaller than normal (Fig. 2).

The cones of type 3 were, in the specimens examined, much smaller than the normal cones for the trees. Usually they were only one-third of the normal size and contained no full seed, the ovules having failed to develop. In this type there were usually one or two female cones surrounded by male strobili. One case was observed, however, in which a cone, situated at the top of a male inflorescence, had produced seed. The seed were apparently normal in every respect except size.

The position of the male strobili on the shoot

Normally the male cones, or strobili, are produced at the extreme base of the shoot. This is the case in both uninodal and binodal shoots, although male shoots are generally uninodal. In no case have male strobili been observed on the top internode of binodal shoots.

The normal male inflorescence is characterized by its position at the extreme base of the shoot, and by the fact that the strobili completely surround the shoot. One abnormality is that in which the inflorescence is separated from the base of the shoot by a large, or small, number of fascicles of needles. Another is that in which the strobili tend to be sporadic, or semi-irregular, in their distribution and do not completely surround the shoot. This abnormality may be subdivided as follows:—

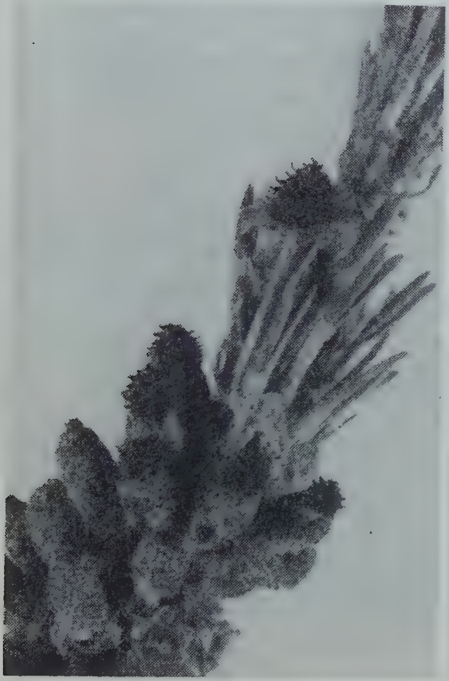

FIG. 3. The upper portion of a male inflorescence which contains bisporangiate strobili. A normal young female cone can be seen at the node above the inflorescence.

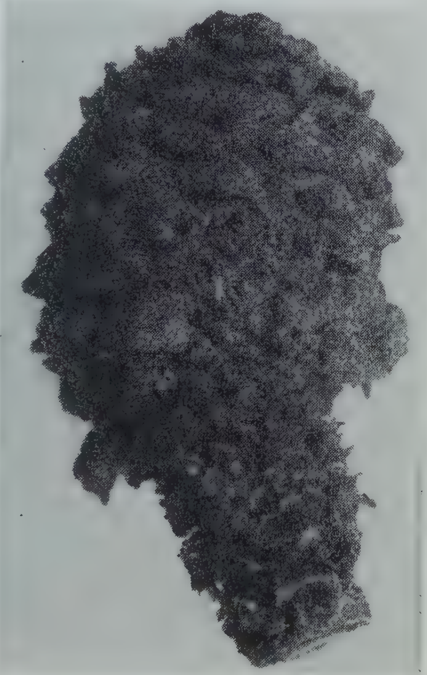

FIG. 4. A mature bisporangiate strobilus, the remnants of the anthers can be distinguished at its base.

(*a*) The inflorescence circumvents half the circumference of the shoot and extends up its length for a more or less normal distance. It may be established at the extreme base of the shoot, or separated from it by fascicles of needles.

(*b*) The strobili are a few in number, four to six being the normal, and are situated in a little cluster on the basal half of the shoot. They give the impression that the male strobili have replaced a small number of fascicles of needles.

In the last description it has been stated that the male strobili give the impression of having replaced fascicles of needles. The reverse is also true. Needles were discovered in the midst of male inflorescences. Usually the number of fascicles of needles present was small, on the average about four to six.

A third, extremely abnormal, type was also found to exist although only one case of it was observed. This occurred on an inland type of tree, in which a single male cone, or strobilus, had replaced the terminal bud on a shoot of low vigour. Apparently, instead of forming needles and elongating in the normal manner, the shoot had produced ten fascicles of needles and then a male cone in the position normally occupied by the terminal bud. This strobilus was typical in size and colour for the tree, and produced, as far as could be ascertained, normal pollen.

Bisporangiate strobili

On trees of inland origin which were examined about the time of anthesis, it was observed that some of the male strobili, or cones, consisted not only of male but female tissue also. The normal young female cones, at the stage examined a few weeks old, on the trees had a reddish-purple colour; the central body of the cone being purple, and the spines of the scales red. The bisexual cones were orange-yellow at the base, the normal colour of the male inflorescence on these trees, and purple at the apex. If the female part of the bisexual cone was large a faint reddish tinge could often be distinguished. This difference in colour was due to the fact that the spines of the ovuliferous scales on the bisexual cones were short and purple in colour. Only if the female part of the bisexual cone was relatively large did the spines attain much size, and with size a discernible reddish hue.

Longitudinal sections were cut through a male strobilus, a female cone

Table 1

Details of the Origins of the Provenances

	Latitude (North)	Longitude (West)	Altitude (ft.)
Terrace	54° 30′	128° 30′	200
Queen Charlotte Islands	52°–54°	131°–133°	S.L.–150
Sonora Island—New Westminster	50° 25′ 49° 12′	125° 20′ 122° 56′	S.L. S.L.
Olympic Peninsula	47°–48°	123°–125°	300–400
Grays Harbour	47° 5′	124°	?
Hazelton	55° 14′	127° 39′	1,150
Telkwa, Smithers	54° 47′	127° 11′	2,000
Prince George	53° 56′	122° 43′	1,867
Shuswap Lake	51°	119°	2,100–2,700
Shuswap, Salmon Arm	50° 42′	119° 18′	1,200
Priest River Valley	48° 20′	116° 15′	2,380
Williamson River, Klamath	42° 30′	122°	3,400–5,000

The first five provenances are of coastal origin, the remainder are of inland origin.

aged two to three weeks, and one of the bisporangiate strobili. The basal part of the bisexual cone was normal when compared with the male strobilus, and the pollen also did not appear to be abnormal in any way. The upper part of the bisexual cone was, in appearance, typically female; the ovules, ovuliferous scales, and bracts being apparently those of a normal, healthy, female cone. (Figs. 3 and 4).

Various facts were noted regarding the occurrences of these bisexual cones.

1. They were found to be present in male inflorescences of various types.
    (*a*) In otherwise normal male inflorescences found on uninodal shoots bearing no female cones.
    (*b*) In male inflorescences situated on binodal shoots having female cones at the middle node. The inflorescence may be separated from the base of the shoot by a large, or small, number of needles or alternatively, the inflorescence may not have needles situated below it. In the latter case, however, occasional needles are found in the male inflorescence giving the impression of having replaced strobili.
    (*c*) In male inflorescences, on binodal shoots having female cones at the middle node, confined to one side of the basal internode; the inflorescence being separated from the base of the shoot by fascicles of needles.
2. In all the cases examined the bisexual cones were found to be present mainly towards the top of the male inflorescence, and seldom below the upper half of the inflorescence.
3. The number of bisexual cones present in a male inflorescence was usually small, and seldom exceeded ten no matter how vigorous the shoot or how large the inflorescence.
4. The male inflorescences containing the bisexual cones were normally found only on shoots situated in, approximately, the upper half of the crown of the tree.
5. One case was observed in which a single bisexual cone occurred halfway along a vegetative shoot. It was completely surrounded by needles and gave the impression of having replaced a single fascicle.

Generally, the proportions of the length of the axis of the bisexual cone occupied by megasporophylls never exceeded a half, and was usually considerably less. In the one male inflorescence it was possible to see bisexual cones in which the female portions varied from a half to a twentieth, or even less. Normally, in such a male inflorescence, the bisexual cones nearest the top of the inflorescence had the highest proportion of female tissue, and those situated lower down had a considerably smaller proportion. However, it does appear to be the case that the size of the female proportion of the axes of bisexual cones is influenced by the vigour of the shoot on which the male inflorescence is established, as well as by the presence or absence of female cones. If the shoot is vigorous, and bears normal female cones, bisexual strobili with a high proportion of female tissue are usually present. On the other hand, lack of vigour and absence of female cones are associated with bisporangiate strobili having a low proportion of female tissue.

In the section dealing with the abnormal production of female cones it was stated that cones were observed in male inflorescences. As described in the preceding paragraphs bisporangiate strobili are also found in male inflorescences. In the few examined specimens of the former abnormality the inflorescence contained female cones and male strobili, but no bisporangiate strobili. However, it can be stated that normally when an inflorescence contains male and female strobili, bisexual cones are also present. The male strobili are situated at the base of the inflorescence, bisexual strobili in the middle, and female cones at the apex. These inflorescences may also have fascicles of needles present in them.

The production of abnormalities within individual trees

Although the variation in the types of abnormalities, and their occurrence, tends to be confusing, certain points are comparatively clear. All the abnormalities involving female cones and bisporangiate strobili were observed on shoots situated in the tops of crowns, never on shaded shoots in the lower portions. This was also true of a high proportion of the abnormalities concerning the male inflorescence. Thus, it may be the case that the production of these abnormalities is influenced, directly or indirectly, by light.

It is curious that bisporangiate strobili have not been observed on binodal shoots with sub-terminal cones. This may be indicative of the fact that their production is influenced in some way by the proximity of female cones. The occurrence of bisporangiate strobili on uninodal male shoots, bearing no female cones, can be attributed to the fact that the terminal bud may well have contained the germ of a female cone.

In the preceding paragraphs it has been stated that the occurrence of abnormalities is probably affected by light and, also, that the proximity of female cones or buds influences the production of bisporangiate strobili. These statements hold for the majority of trees examined but there is one notable exception. This tree of inland origin, situated on the Lon Mor, Inchnacardoch Forest, Inverness-shire, exhibits most of the described abnormalities, e.g. replacement of male by female strobili, bisexual strobili, &c. The vast majority of the shoots of this tree have some type of abnormality, in comparison to the usual condition where the abnormalities are comparatively scarce. In this case the abnormalities may be seen on shoots of low vigour, and the presence of normal female cones apparently does not influence the production of bisporangiate strobili.

DISCUSSION

It is clear from the above information that fascicles of needles and male and female strobili appear to be, to some extent, interchangeable. For example, female may replace male strobili, and needles male strobili. These phenomena have been recorded by other workers. Jackson (1928) reports that *Pinus thunbergii* Parl., in Britain has produced large clusters of cones, and from his illustrations it appears to be the case that female have replaced male strobili. Zobel (1952) records that female cones have replaced fascicles of needles on *Pinus ponderosa* Douglas.

Bisexual strobili have been found in *Abies*, *Picea*, *Pseudotsuga*, *Sequoia*, and *Juniperus* as well as in *Pinus* (Coulter and Chamberlain, 1910; Masters, 1890; Righter, 1932). Katayama and Shida (1953) found that the frequency of flowers intermediate to the female and typical intersexual types of a Japanese variety of spinach rose with decreasing photoperiod. Roberts and Struckmeyer (1939) state that maize at temperatures of 60°–65° F. in long days frequently produce androgynous tassels and poor ears. In short-day conditions abortive tassels are produced but the ears are more normal in appearance. Borthwick and Scully (1954) showed that temperature and light influence the production of male flowers on female plants of hemp. Thus, it appears that both temperature and light affect the production of abnormal flowers and inflorescences.

Sorauer (1909) states that the most common abnormality in pines is that in which female replace male strobili. He attributes it to 'a local surplus of concentrated formative substance'. Sauer (1954) examined catkins of a hermaphrodite tree of *Populus tremula* L. and found androgynous flowers present in some of them. They were usually situated at the apex of the catkin. He concluded that the abnormality may be influenced by the age of the tree and is the result of genic lability, especially in hybrids.

Zobel (1952) states that abnormal cone formation often appears to be caused by abrupt physiological upsets due to a shock of some kind, e.g. severe pruning, fertilizing, irrigating, or planting out of the species' natural range. This shock would thus appear to have some effect upon the genetic make-up of the plant resulting in these changes, e.g. bisporangiate strobili. One evident case of shock is recorded by Borggreve (1880) in which several fifteen-year-old spruces were transplanted. In the following year the terminal shoots of the trees bore female inflorescences.

Zobel's (1952) point regarding planting the species out of its natural range appears to be sound when the literature is examined. In the cases reported for *Pinus ponderosa* Douglas, *P. thunbergii* Parl., *P. massoniana* Lambert, and *P. densiflora* Siebold and Zuccarini all the trees were out of their natural range (Zobel, 1952; Jackson, 1928; Righter, 1932). A case concerning *Populus candicans* Aiton (Sauer, 1954) is more doubtful as its native habitat is not certain (Rehder, 1940). However, the abnormalities reported by Sauer (1954) occurred on a tree in Germany and the species may have originated from North America (Bean, 1950–1). Abnormalities have also been reported on *Populus alba* L., as well as *Populus tremula* L. (Sauer, 1954), in Germany which is within their natural range. However, the trees examined may well have suffered a shock of some other type, e.g. planting on an unsuitable site.

All the abnormalities described in *Pinus contorta* Loudon occurred, and were fairly common, on trees of inland origin. Only one tree, in all the coastal provenances examined, exhibited an abnormality. This was a specimen of the formation of cone clusters, i.e. the replacement of male by female strobili. This is not surprising in view of Zobel's (1952) statement. The trees of coastal origin came from a maritime climate not unlike that of Great Britain, while the inland provenances are from a more continental climate.

The presence of abnormalities on a tree may be taken to indicate that it has suffered a shock. This is probably due, in most cases, to an unsuitable environment. If this is so then a tree which has abnormalities will probably not be as suited to its environment as one from which they are absent. It would thus appear to be the case that if trees having these abnormalities were removed, the population, as a whole, would become more suited to its environment.

In conclusion it can be stated that the prevalence of abnormalities in the reproductive system of the inland provenances is probably indicative of the fact that they are not well suited to growing conditions at Millbuie. On the other hand, the absence of such abnormalities from the coastal provenances indicates that they are better adapted to the environment on this site.

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Effects of Age and Environment on Net Assimilation Rate of Barley

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With three Figures in the Text

ABSTRACT

To distinguish between the effects of age and environment on net assimilation rate (E) of barley (var. Brant) grown in the open in pots at Ottawa, E was measured outdoors (treatment N) and on similar plants transferred to a constant environment for the 2 weeks during which E was determined (treatment T). During June and July, E of treatment N decreased by 77 per cent.; for treatment T this fall increased to 90 per cent. of the initial value. E and average day and night temperatures were greater in the constant environment than outdoors in early June, and in July they were greater outdoors. Incident light energy was always greater outdoors. Thus, the fall in E with age was partially masked outdoors by an increase caused by the improvement in environmental conditions during the experiment.

Plants grown continuously in the constant environment had lower E, greater leaf area and dry weight, and ears emerged earlier than plants of similar age that had been transferred for 2 weeks to the constant environment from outdoors. E of plants grown continuously in the constant environment decreased with time, both when the pots were moved away from the light panel to maintain a constant light intensity at the base of the youngest leaf and when the pots remained stationary so that the plants grew towards the lights.

After ears emerged, E for treatments N and T was measured on plants whose ears were shaded to prevent them photosynthesizing. Photosynthesis in the ear accounted for 19 per cent. of the final ear dry weight during the first 2 weeks after emergence, 12 per cent. during the 3rd and 4th, and 3 per cent. during the 5th and 6th weeks. Shading had no effect on weight of plant parts other than ears and had similar effects outdoors and in the constant environment.

INTRODUCTION

NET assimilation rates (E) of plants grown in the open as crops or in pots show smooth time trends that are usually attributed to changes in environmental factors; E varies from high values in mid-summer to low values in winter (Watson, 1952). E may be affected by age as well as by seasonal trends in external factors, but the two effects are difficult to distinguish, especially as any decrease in E with age will usually occur concurrently with the decrease brought about by the seasonal fall in daily radiation and temperature. When net assimilation rates of plants sown at different dates were compared at the same time, there was little or no variation with age compared with the seasonal trend (Watson, 1947; Blackman and Wilson, 1951). However, when seasonal fluctuations in the environment were eliminated, by growing the plants in growth-rooms, E fell by as much as 15 per cent. per week (Thorne, 1960).

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To determine how much of the variation in E of barley grown in the open is caused by change in the environment, E was measured simultaneously throughout the season on plants grown in the open and on similar plants transferred to a controlled environment for the period during which E was estimated. E of plants grown continuously in the controlled environment was also measured, to see how it is affected by variation in conditions before the interval when it is determined.

METHODS

The experiment was done in 1958 at Ottawa, Canada. Barley (var. Brant) was sown on 16 May in 1 gallon glazed pots filled with soil. The 144 pots, each containing 6 plants, were divided between the following environments:

N. Natural conditions in the open, but protected from rain.
H. Growth-room with 18 hours of high light intensity (1,800 f.c.) at 20° C. and 6 hours of darkness at 15° C.
L. As H, but with lower light intensity (800–1,200 f.c.).

Plants were grown as described previously (Thorne, 1960). Treatment H and Expt. 3 of the earlier paper are identical. For treatment H, light intensity at the base of the youngest leaf was maintained at 1,800 f.c. by increasing the distance between the pots and the light panel as the plants grew. Hence the average intensity of light falling on the leaves decreased during the experiment. Such a decrease, which might cause some of the fall in E observed in these conditions (Thorne, 1960), was avoided in treatment L. Light intensity at the top of each pot was 800 f.c. and the pots were not moved away from the light, so the average intensity of light falling on the leaves increased as the plants increased in height.

Eight pots of treatment N were sampled on 7 occasions at fortnightly intervals. The first (week 1) was 18 days after sowing and the last (week 13) was at maturity. On each of the first 5 occasions, 8 similar pots (treatment T) were transferred to conditions identical with treatment H and harvested 2 weeks later. At the first 2 sampling occasions after ear emergence, an additional 8 pots were transferred to the growth-room after all the emerged ears had been covered with paper shades to prevent them photosynthesizing (Watson, Thorne, and French, 1958). Ears that emerged after transfer were shaded as they appeared. Ears of 8 N pots that were to be harvested at the next sampling were also shaded in the same manner and ears of 8 N pots to be harvested at maturity were shaded from emergence onwards. Eight pots were sampled from treatments H and L on 4 occasions at fortnightly intervals, starting 25 days after sowing, i.e. at weeks 2, 4, 6, and 8, the alternate weeks to those in which treatments N and T were sampled.

Each pot was allocated to one of the 8 replicates in the growth-room according to average light intensity before sampling started and outdoors according to the degree of shading by nearby buildings. Blocks receiving the lowest and highest intensity of light in the growth-room were associated with those outdoors having most and least shading respectively.

Growth attributes were calculated for each 2-week interval between samplings (Thorne, 1960). For treatment T, growth during the interval was obtained from the difference between T plants at the end of the interval and N plants at the beginning of the interval. After ear emergence E was calculated from the increase in dry weight of plants with shaded ears, which was attributable entirely to assimilation in the leaves. The difference between dry-weight increments of shaded and unshaded plants measured the amount of assimilation occurring in the ears.

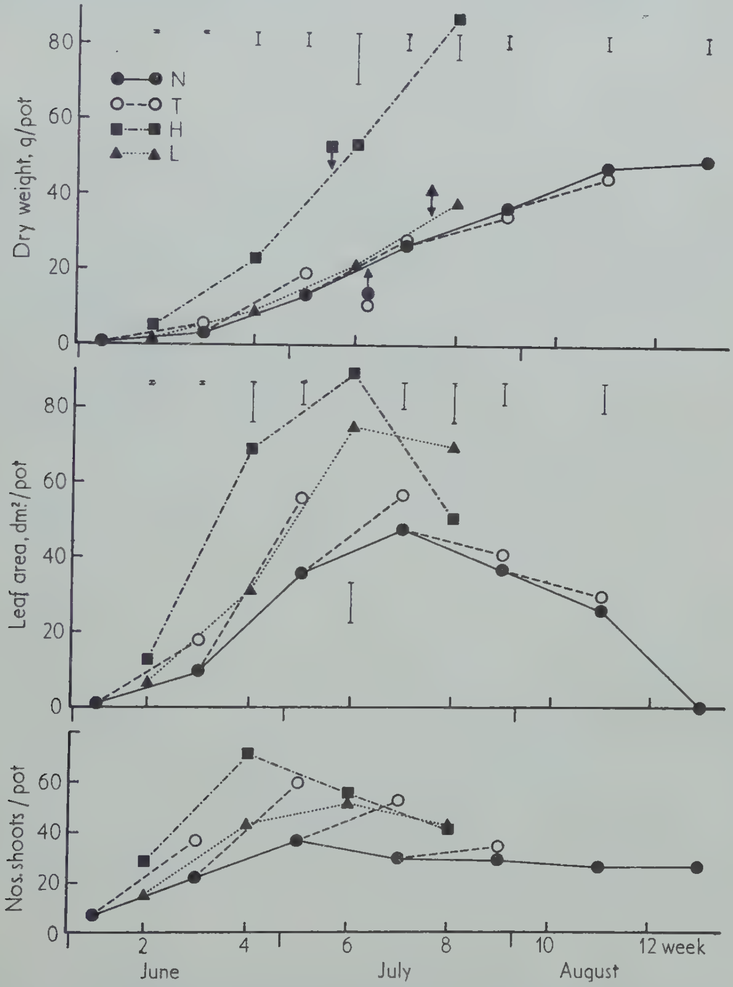

FIG. 1. Dry weight, leaf area, and shoot number of pots of 6 barley plants grown continuously outdoors (N), grown outdoors and transferred to the growth-room for 2 weeks (T), grown continuously in the growth-room with a high light intensity (H) or a low light intensity (L). Vertical lines in this and subsequent figures represent least significant differences ($P = 0{\cdot}05$) and the arrows indicate the mean date of ear emergence.

Average day and night temperatures and the amount of light received by the plants were measured to relate variations in growth with environmental differences. In the growth-room, temperature and light intensity were measured with thermocouples and a Weston light meter (Thorne, 1960). Mean day and night temperatures outdoors (treatment N) were obtained by averaging readings from thermograph charts over periods separated by sunrise and sunset. The amount of light received by plants of treatment N was measured with a Kipp solarimeter, fitted with a Corning 1–69 filter that transmits only between 300 and 760 mμ, attached to an integrating recorder. The Weston light meter was calibrated against the solarimeter in the growth-room to measure in energy units the amount of light received by plants of all treatments.

RESULTS

Dry weight, leaf area, and net assimilation rate

At weeks 3 and 5 the plants transferred to the growth-room from outdoors 2 weeks previously (treatment T) had a greater dry weight than similar plants that remained outdoors (treatment N) (Fig. 1). Subsequently, the dry-weight increments of N and T plants did not differ significantly. Transfer to the growth-room increased leaf area compared with N at all samplings and increased shoot number at all except week 11 (Fig. 1). This increase was apparently caused by a greater rate of shoot production for T than for N, rather than by delayed senescence; both before and after the time of maximum leaf area and shoot number, transferring to the growth-room increased the number of small shoots but had little effect on the number with areas over 60 cm^2.

Net assimilation rate was greater for T than for N during weeks 1–5 and subsequently was slightly greater for N (Fig. 2). It decreased steadily with time, outdoors by 77 per cent. in 8 weeks and in constant conditions (treatment T) by 90 per cent. Leaf-area ratio (F) was increased slightly by transfer to the growth-room after week 3 and relative growth-rate (R_W) was increased between weeks 1 and 5.

Plants grown continuously in the controlled environment with the high light intensity (treatment H) always had a greater dry weight than plants with other treatments (Fig. 1). Leaf area and shoot number were also greater for H than for L, N, or T, except at week 8 when leaves and shoots of H plants had started to senesce (Fig. 1). At the lower light intensity (L) dry weight at all samplings, and leaf area and shoot number at weeks 2 and 4, were less than for H and similar to T. At week 8 leaf area and shoot number were bigger for L than for the other treatments.

Net assimilation rate of plants of treatment H between weeks 2 and 6 was less than that of plants of the same age, previously grown outdoors, measured in the same environment (T) or measured outdoors (N). The rate was less for plants of treatment L than for H. It decreased significantly between the first 2 intervals for both treatment H and L, and increased between the second and third intervals slightly for L and considerably for H. This increase saw

TABLE 1

Average Values for Fortnightly Intervals between Samplings of Leaf-area Ratio (cm²/g.) and Relative Growth-rate (per cent. per week) for Treatments N, T, H, and L

	Leaf-area ratio (F)				Relative leaf growth-rate (R_W)			
Week	N	T	H	L	N	T	H	L
1–3	280	273	—	—	98	133	—	—
2–4	—	—	286	344	—	—	75	72
3–5	306	321	—	—	72	93	—	—
4–6	—	—	222	392	—	—	41	62
5–7	215	247	—	—	38	40	—	—
6–8	—	—	100	271	—	—	34	44
7–9*	132	143	—	—	19	16	—	—
9–11*	70	78	—	—	5	−2	—	—
S.E.	6.5		12.9		2.9		3.3	

* Plants with shaded ears.

probably a result of attributing dry matter that had been assimilated by the ears to assimilation by the leaves; ears of treatment H started to emerge at week 5 and of treatment L at week 7 and, unlike treatments N and T, none were shaded. Ears of treatments N and T started emerging at week 6.

Leaf-area ratio for treatment H was similar to N and T between weeks 2 and 4, and thereafter slightly smaller (Table 1). L plants always had the greatest leaf-area ratio. Relative growth-rate of plants of treatment H was less than for any other treatment during the experimental period, although these plants had the greatest total dry weight.

The distribution of dry matter within the plant did not differ between treatments except when rate of development was affected.

Effect of shading ears

Shading the ears for 2 weeks before the samplings at weeks 9 and 11 decreased dry weight of the ears of N and T plants (Fig. 3), slightly more, but not significantly so, with T than N. Dry weight of other parts of the plant was unaffected. Shading the ears of N plants for the 2 weeks between week 11 and maturity decreased dry weight slightly further. Photosynthesis in the ear of N plants, estimated from the differences in weight of shaded and unshaded ears, contributed 19 per cent. of the final ear weight during weeks 7–9, 12 per cent. during weeks 9–11, and 3 per cent. during weeks 11–13. Shading ears continuously between weeks 7 and 13 decreased ear weight by 38 per cent. and grain weight by 43 per cent.

Environmental conditions

The quantity of light was always greatest for N and least for L (Fig. 2). It fell slightly after ear emergence for treatments T and H because elongation of the peduncle made it impossible to maintain an intensity of 1,800 f.c. at the base of the flag leaf. Light intensity for treatment L increased slightly during the experiment but never reached that of treatment H because the L plants did not attain the same height as those of treatment H. The natural day length increased from 15 hrs. 30 min. at week 1 to a maximum of 15 hrs. 42 min. and decreased to 14 hrs. 18 min. at week 11. The hours of bright sunshine varied similarly to the quantity of light.

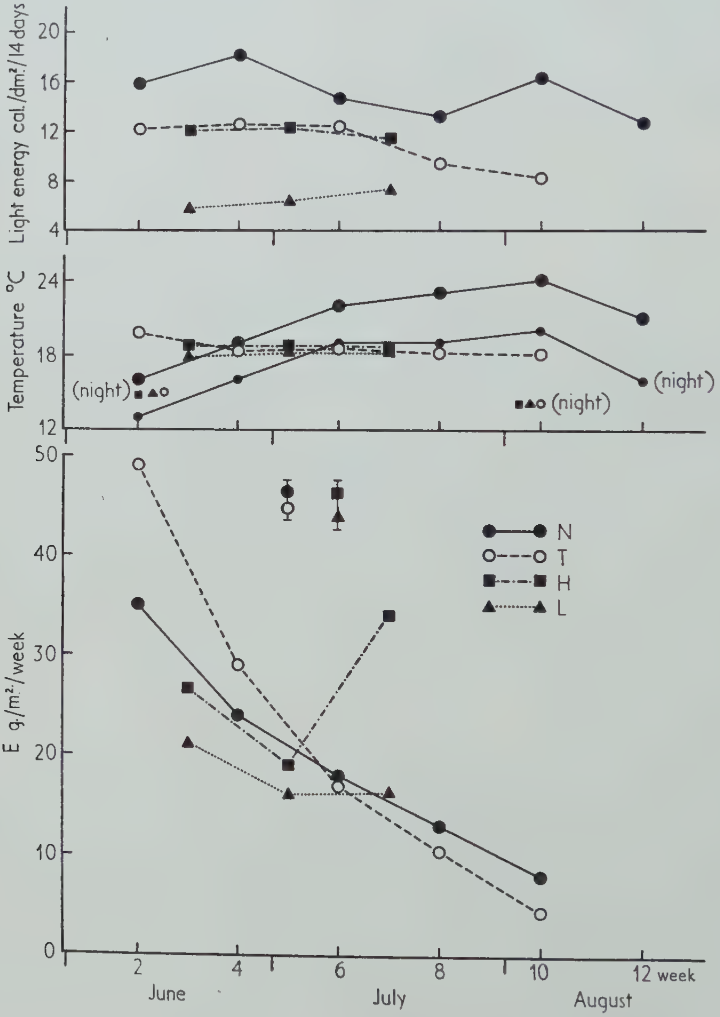

FIG. 2. Quantity of light, mean day and night temperatures, and net assimilation rate (E) of barley measured over fortnightly intervals. Treatments N, T, H, and L as in Fig. 1.

Average day and night temperatures outdoors (N) were lower than in the growth-room for the first 2 weeks and thereafter higher. Temperatures in the growth-room were similar for all treatments (T, H, L) and were almost constant throughout the experiment (Fig. 2).

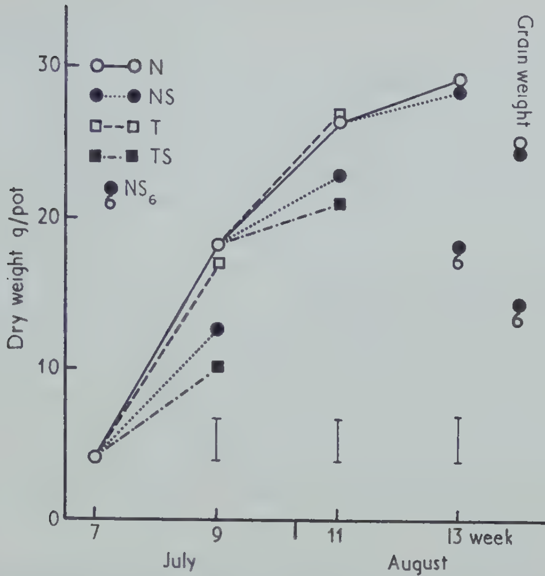

FIG. 3. Ear and grain dry weight of pots of 6 barley plants grown outdoors with ears shaded for 2 weeks (NS), shaded for 6 weeks (NS_6), or unshaded (N); or transferred to growth-room for 2 weeks with shaded ears (TS) or unshaded ears (T).

DISCUSSION

Net assimilation rate of barley decreased with time both in the natural variable environment (N) and in the constant environments (T, H, L), confirming the conclusion reached previously that E of barley falls with age independently of the environment (Thorne, 1960). The rate of fall for treatment T decreased throughout the experiment from 20 per cent. of the initial value per week in mid-June to 6 per cent. in early August. The mean rate of decrease up to ear emergence of 16 per cent. of the initial value per week was similar to that found for this interval for plants grown continuously in the growth-room (Thorne, 1960).

The smooth time trend in E of the plants grown outdoors (N) in this experiment was not caused by changes in environmental factors. On the contrary, environmental conditions improved with time so that the fall in E with age was partially masked. The greater E for treatment T than for N at week 2 was probably caused by higher temperature in the growth-room. The quantity of incident light energy was always greater outdoors. Both higher temperature and more light may have caused the slightly greater E outdoors at the end of the experiment (Fig. 2). The data are not suitable for determining how environmental differences brought about differences in E between treatments N and T because E and environmental factors drifted smoothly with time in the growth-room and outdoors, in a manner unlike that usually observed in England, where fluctuations in E and in environmental factors averaged over weekly or fortnightly intervals may be large (Gregory, 1926; Watson, 1947).

There was no initial rise in E of barley outdoors as has been reported from England (Watson, 1947) because of the late sowing (16 May) necessitated by the late Canadian spring, and because E was based on leaf area that included sheath as well as lamina. The proportion of the total area accounted for by sheath increases from zero shortly after germination to about 50 per cent. at ear emergence, and when Watson's curve of E against time is redrawn allowing for sheaths, E falls continuously between mid-May and early July as in Canada.

Unlike dry weight and E, which were greater for T than for N at the beginning of the experiment and less at the end, shoot number and consequently leaf area were increased by transfer to the growth-room at all times (Fig. 1). The cause of this increased shoot production is unknown. It was caused neither by the difference in temperature, whose direction reversed during the experiment, nor by the consistently lower light intensity for T than for N, because tillering is normally favoured by high light intensity (Kamel, 1959). It is unlikely to have been caused by the greater uptake of nitrogen by T plants which occurred only before week 5 (Table 2). This extra N uptake was probably caused by the increase in dry weight; N per cent. of dry matter was unaffected. The high relative humidity in the growth-room of approximately 70 per cent. may have affected shoot production. Relative humidity outdoors usually fell below 50 per cent. during the day, but was near 70 per cent. at night. However, there is little evidence that humidity affects growth of plants given enough water (Went, 1957) and, as all the plants were well watered, it is doubtful whether the high humidity in the growth-room caused the increased shoot production.

Net assimilation rate of plants in a growth-room may decrease with time when a constant light intensity is maintained near the tip because the average intensity received by all the leaves falls as the plants increase in height (Thorne, 1960). This was not the only cause of the decrease with time in E of plants of treatments T and H, because E also decreased up to ear emergence for treatment L, in which the plants grew towards the light panel from a fixed position (Fig. 2). The low E of treatment L was presumably caused by the low light intensity which also increased F (Table 1), but this was insufficient to compensate for the decrease in E, and total dry weight was less for L than for H. The higher R_W for L than for H during weeks 4–8 probably reflected the

TABLE 2

Nitrogen Content of Whole Plants of Treatments N and T

	N mg./pot		N % D.W.	
Week	N	T	N	T
3	175	373	6·2	6·5
5	626	886	4·8	4·7
7	909	998	3·4	3·6
9*	1,050	1,082	2·5	2·5

* Plants with unshaded ears.

slower development and hence slower fall of R_W with time at the low than at the high light intensity.

Net assimilation rate was affected by the previous history of the plant. It was always greater for treatment T than for H, though measured in the same conditions on plants of the same age. Self-shading among the more numerous leaves in treatment H may have decreased the average intensity of light they received, and hence decreased E compared with treatment T. Such a mechanism could account for E of treatment H at week 3 being similar to that of T at week 5, as their respective leaf areas at these times were also similar. But, although E of treatment H at week 5 was similar to that of T at week 6, leaf area for H was almost twice that for T.

Another possible explanation for the low E of treatment H after week 5 is high respiration loss associated with the low leaf-area ratio (F). But at week 3 there was also a difference in E though the values of F were almost identical. The difference between treatments in F probably reflected the faster development of plants of treatment H, whose ears started emerging at week 5, 1 week before those of treatment T. The developmental stage of the plant, rather than its absolute age, probably also affected whatever other factors caused E to decrease with age, so it is reasonable to expect E of the more mature plants of treatment H to be smaller than for treatment T. The differences in E observed during the experimental period are consistent with a difference in developmental age of 1 week: E of treatment H at weeks 3 and 5 did not differ significantly from that of treatment T at weeks 4 and 6 (Fig. 2). But this difference of 1 week in 'physiological age' cannot occur right back to sowing. The lack of data for the first 4 weeks after sowing, and the variable environmental conditions experienced by plants of treatment T before the period when E was measured, prevent a more detailed analysis of how previous history affected E of similar aged plants when E was measured in identical conditions.

Conditions during the first 18 days after sowing, i.e. before week 1, had a profound effect on leaf area and dry weight as well as on E. At week 2 leaf area and dry weight were much greater for treatment H than for N, and, although R_W after week 2 was less for H than for N, this difference was maintained and would have persisted to maturity; ear dry weight for H at week 8 was 4 g. greater and total dry weight was 37 g. greater than the final weights for treatment N. Summing the effects on dry weight of transfer to the growth-room for each fortnightly interval indicates that, if plants from outdoors had been kept in the growth-room continuously from week 1 to maturity, their final dry weight would have been about 10 g. greater than that of treatment N. So if plants grown outdoors or in the growth-room until week 1 had then both been grown in the growth-room until maturity their total dry weights would have differed by about 27 g. Thus, favourable conditions during the first 18 days after sowing could increase final dry weight by 45 per cent. Average conditions outdoors before week 1 were similar to those for the period between weeks 1 and 3, i.e. there was about 30 per cent. more light than in the growth-room and temperatures were 4–5° C. lower. More experiments are required to determine whether the early difference in temperature caused the persistent difference in dry weight, and if so, what mechanism was involved.

Photosynthesis in the ear, as measured by shading ears of treatment N, accounted for 38 per cent. of the final dry weight. This is greater than for 2-rowed barleys, presumably because there is more assimilation from the larger surface area of the 6-rowed ear. In spite of the lower light intensity, ears consistently assimilated more in the growth-room than outdoors, though the difference was not quite significant (Fig. 3). This may have been because respiration loss was decreased by the lower temperature in the growth-room. Ear weight is probably more sensitive to temperature changes than leaf weight because respiration rates of ears are higher compared with their rate of photosynthesis; measurements with an infra-red gas analyser have shown that respiration rate of ears is sometimes more than half their rate of true photosynthesis. The rate of photosynthesis of the ear decreased with time so that 56 per cent. of the total assimilation by the ear occurred in the first 2 weeks after emergence, 35 per cent. in the next 2, and 9 per cent. in the last 2. This agrees closely with the percentages of 60, 33, and 7 for successive 2-week intervals obtained by direct photosynthesis measurements on a 2-rowed barley (Porter, Pal, and Martin, 1950).

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Investigations into the Stomatal Physiology of Upland Cotton

I. The Effects of Hour of Day, Solar Radiation, Temperature and Leaf Water-Content on Stomatal Behaviour

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With four figures in the text

ABSTRACT

Stomatal behaviour was studied under field conditions using an infiltration method with solutions of *iso*-butanol diluted with ethylene glycol. For mature leaves not suffering water strain, stomata in both epidermes commence opening at sunrise (6.30 a.m. local time) and have a maximum aperture between 10 a.m. and 3 p.m.; subsequently a slow closure commences and this is complete by 7 p.m. For leaves suffering marked water stress, i.e. with relative turgidity values falling below 85 per cent., the period of maximum aperture is curtailed by a varying amount dependent upon the extent of water stress; closure occurs earlier, and no evidence has been found for a second opening later in the day.

For leaves not suffering water strain it is shown by means of a multiple correlation analysis that stomatal aperture is significantly correlated with solar radiation, temperature, and hour of day. It is suggested that the hour-of-day effect may be a true diurnal rhythm controlled by factors within the leaf. The correlations noted above are not found for leaves with low values of relative turgidity, since the effects of water strain tend to over-ride those due to the other factors.

INTRODUCTION

THE stomatal behaviour of cotton has been investigated by a number of authors. Balls (1912) studied the behaviour of stomata in *Gossypium barbadense* using a recording porometer. Lloyd (1913) investigated the relationship between stomatal movement and leaf-water content for *G. herbaceum* using a technique involving direct observation of the stomatal pore. Weindling (1948) used a simple injection technique to study stomatal behaviour in *G. hirsutum*, in connexion with studies on infection of leaves by *Xanthomonas malvacearum*. No data have hitherto been presented on the stomatal behaviour of *G. hirsutum* grown under East African conditions although there has been considerable interest in the water relations of cotton (Weatherley, 1950; Hutchinson, Manning, and Farbrother, 1958).

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The present study, which has been restricted to cotton grown under field conditions at Namulonge and at Ukiriguru in Lake Province, Tanganyika, represents an investigation into the effect of four factors known to influence stomatal behaviour, and the relative importance of these factors has been assessed using multiple correlation analyses.

Other data, from experiments performed under laboratory conditions, will be presented in a later communication.

MATERIALS

Most of this work was carried out using the Upland cotton variety BP52, stomatal dimensions of which are as follows:

Length of guard cells	25–40 μ
Length of aperture	10–15 μ
Width of guard cell pair including aperture	20–30 μ
Width of aperture	0–6 μ
Maximum area of pore (assuming it to be an ellipse)	$7{\cdot}0\times10^{-5}$ mm.^{2}

Dimensions of the guard cells were found to be unaffected by position within or between leaves.

Stomatal frequency for mature leaves of BP52 varies between limits depending upon position within the leaf, leaf position on the plant, season, and (it is suspected) locality. The range of values found for samples taken from between the main veins of mature leaves from the 10th to the 15th node on the main stem of rain-grown cotton were as follows:

Upper epidermis: 37·6–50·3 stomata per mm.^{2}
Lower epidermis: 107·3–120·6 ,, ,, ,,

Assuming values of 50 and 120 stomata per mm.^{2} for the two surfaces, and a maximum pore area per stoma of $7{\cdot}0\times10^{-5}$ mm.^{2}, the maximum pore area per mm.^{2} is:

Upper epidermis: $3{\cdot}5\times10^{-3}$ mm.^{2} (0·35%)
Lower epidermis: 8.4×10^{-3} mm.^{2} (0·84%)

Similar values of size and frequency were found for the variety Uk55 which was used in the observations made at Ukiriguru.

METHODS

Stomatal aperture. This was estimated by an infiltration method using *iso*-butanol diluted with various amounts of ethylene glycol (Schorn, 1929). Ten solutions ranging from 100 per cent. *iso*-butanol, which penetrated stomata of small aperture, to 10 per cent. *iso*-butanol+90 per cent. ethylene glycol, which penetrated only those stomata with very wide apertures, were used. Results are expressed in terms of numbers of solutions penetrating the leaf and for convenience this is referred to as the Infiltration Score (I); a score of 1 represents injection by 100 per cent. *iso*-butanol only, and a score of 8 represents injection by all solutions down to 30 per cent. *iso*-butanol+70 per cent.

ethylene glycol. Determinations were generally made on a sample of 10 mature leaves borne at the 4th node down the main stem of similar plants. The area in the middle of the leaf and between the main veins was used.

To check that the method estimated stomatal aperture, an alternative method involving microscopic observation of stomata was used. Two difficulties were experienced;

1. Using transmitted light, assessment of pore width may be made accurately, but the high light intensity necessary caused stomatal movement to occur during the period of observation. Lloyd (1913) does not appear to have experienced this difficulty working with *G. herbaceum*.

2. With reflected light and the objectives available, it was not possible to measure pore width to within 3–4 μ. Since pore width is never greater than 6 μ and generally less than 4 μ, this method is unsatisfactory.

The method finally adopted was that of observation of intact leaves using transmitted light. Ten stomata per sample were rapidly scored as 'shut', 'partly open', or 'open', and *I* was then determined. The grades were assigned arbitrary values of 1, 2, and 3 units respectively and the total value for ten stomata obtained. Comparisons from this somewhat subjective analysis showed that there was a significant linear relationship ($r = +0{\cdot}875$) between the visual estimate of aperture and *I*. Considering that the infiltration method gives an estimate of the largest aperture, and, as used in these experiments, does not integrate the values for large numbers of stomata with different apertures, the correlation may be considered a satisfactory indication that *I* does relate to actual aperture.

Examples of the variation in *I* within and between determinations are given in Table 1.

TABLE 1

The Infiltration Score (as a mean for 10 leaves) and Range, for Stomata in the Upper and Lower Epidermis of Leaves Sampled at Hourly Intervals on 14.9.59

	Upper epidermis		Lower epidermis	
Hour	Infiltration score	Range	Infiltration score	Range
9 a.m.	2.5	2–3	—	—
10 ,,	4.3	3–6	6·0	3–7
11 ,,	5.0	4–6	6·8	6–7
12 noon	5.0	4–6	7·0	6–8
1 p.m.	5.5	5–6	7·0	7
2 ,,	5.6	4–7	7·0	7
3 ,,	4.4	3–6	6·2	5–7
4 ,,	3.2	2–4	6·0	5–7

It was noted that values of *I* were not normally distributed and for the calculation of standard errors a $\log(x+1)$ transformation was used. In general, differences between means of from 0·6 to 0·8 of a unit can be considered significant at the 5 per cent. level.

It will be noted that a smaller score was obtained for stomata in the upper epidermis than for those in the lower. This is a general finding and from direct observation it seems that slight differences in pore width do occur between the two surfaces at any one time (provided the stomata are open). The scores for stomata in the two epidermes are also probably affected by differences in stomatal frequency and leaf structure. The presence of a dense palisade layer in cotton leaves means that there are few large intercellular spaces for the infiltrating agent to inject from the upper surface. Scoring thus becomes rather less accurate than for the lower surface where the volume of penetrable spaces between the mesophyll cells is greater and injection may be more easily observed.

Leaf-water status. This was determined using a relative turgidity method, differing from that due to Weatherley (1950) in that only a single sample of leaf disks was used for each determination. Weatherley's method entailed assessment of dry weight before and after floating on water using duplicate samples of disks. In a series of comparisons it was found that Weatherley's method gave estimates that were about 2·5 per cent. lower than those which did not take account of dry weight losses during floating. It was, however, found to be more convenient to use a single sample of disks and the procedure finally adopted was as follows; 2 disks were punched from between the main veins of each of 10 leaves and the initial fresh weight of the 20 disks was determined ($F.W._{I}$). The disks were then floated on distilled water for 22 hours after which they were blotted and weighed again ($F.W._{F}$), prior to drying for 24 hours at 100° C. The dry weight was determined ($D.W._{F}$), and relative turgidity calculated from the equation:

$$R.T. = \frac{(F.W._{I} - D.W._{F})}{(F.W._{F} - D.W._{F})} \times 100$$

Usually the same leaves were used for the determination of both relative turgidity and stomatal aperture.

Solar radiation. This was recorded using a Kipp solarimeter connected to a Kent 'Multelec' recorder calibrated in g.cal./cm.^{2}/min., and fitted with a mechanical integrating device. Some difficulty arose in the choice of suitable measurements of solar radiation to equate with stomatal aperture. It was considered that 'spot' readings would not be suitable because of wide and rapid variations in the rate of incoming radiation. (Changes in rate of from 0·8 to 1·4 g.cal./cm.^{2}/min. within a minute are not unusual during the mid-day period at Namulonge.) The method used was to measure solar radiation over the 15-minute periods preceding and during the stomatal observations. In this way ephemeral variations in the rate of radiation were smoothed over.

Temperature. Ambient shade temperature was measured using a Casella recording thermograph in a standard screen nine inches above ground level and adjacent to the crop under study. In one series of experiments leaf temperature was measured using thermocouples constructed of 40-gauge copper and constantan wire, with the junctions lightly soldered with silver.

Temperatures were measured to the nearest 0·1° C., which represented 1 unit deflection on a Cambridge Spot galvanometer.

RESULTS

Variations with time of stomatal aperture, relative turgidity, temperature, and solar radiation for two occasions are shown in Fig. 1.

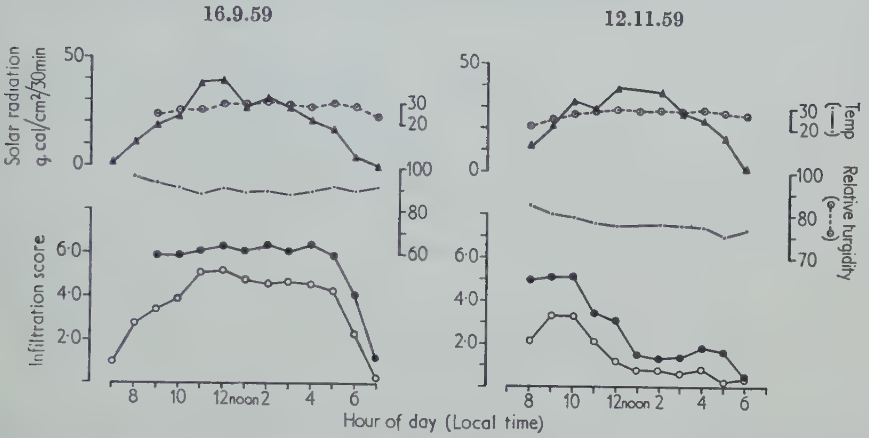

FIG. 1. Infiltration scores for stomata in the upper (open circles) and lower epidermis (closed circles) at hourly intervals on two occasions. Data on solar radiation, temperature, and relative turgidity are also given.

Observations on 16.9.59. In order to reduce dew on the leaves, plants were covered with cloth bags at dusk on 15.9.59. These were removed at dawn (6.30 a.m.) on 16.9.59 when it was found that, although the upper leaf surfaces were free from dew, the lower surfaces were still moist and remained so until about 8.30 a.m. Stomatal observations were therefore restricted to the upper surfaces of the leaves until 9 a.m., when observations were made on both leaf surfaces. Relative turgidity determinations were also commenced at this time. The lowest value of relative turgidity recorded during the day was 87·8 per cent., and the mean value over the period 9 a.m.–7 p.m. was 89·3 per cent. A certain amount of cloud was noted between 7 and 10 a.m. but the period from 10 to 12 noon was clear. Subsequently heavy cumulus cloud began to appear but by 4 p.m. this had dispersed and the evening was clear except for small amounts of very high cloud. The maximum temperature (31° C.) occurred at 2 p.m. Stomatal aperture in the lower epidermis was at a maximum from 9 a.m. until 5 p.m. after which a rapid closure occurred. Maximum aperture for stomata in the upper surface was noted between 11 a.m. and 5 p.m. All stomata were completely shut by 7.30 p.m. There was no evidence of a midday closure of stomata.

Observations on 12.11.59. Plants were covered with cloth bags as before. The light dew had dried by 8 a.m. and all observations were commenced at this time. Relative turgidity was considerably lower than on the previous occasion; the lowest value recorded was 69·8 per cent. and the mean value over the period 8 a.m. to 6 p.m. was 77·4 per cent. The levels of solar radiation and temperature were similar to those on the previous occasion. Stomatal aperture differed markedly however; maximum aperture was observed only between 9 a.m. and 10 a.m., after which time a slow closure occurred, from which there was no recovery later in the day. This closure was thought to be associated with low levels of relative turgidity. Observations on Uk55 cotton at Ukiriguru, made over a period when plants were showing signs of water strain, showed that closure occurred progressively earlier as the level of relative turgidity declined:

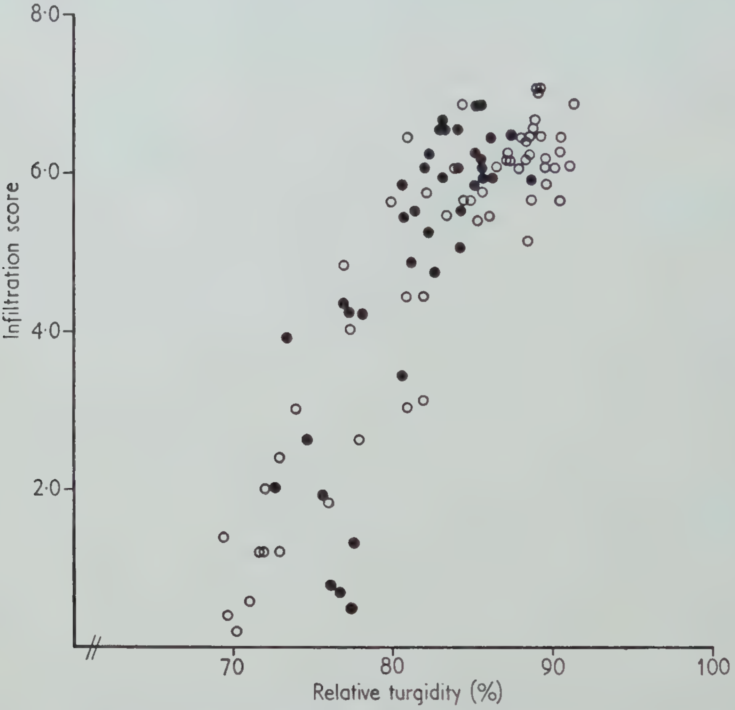

FIG. 2. The relationship between relative turgidity and infiltration score for plants at Namulonge (open circles) and Ukiriguru (closed circles).

	Lowest R.T.	Mean R.T.	Time at which closure commenced
9.3.59	79·2	83·8	4 p.m.
10.3.59	78·2	81·1	2 p.m.
13.3.59	76·5	78·3	11 a.m.

These data suggest that results in Fig. 1 are examples of extreme cases; on the one hand, no water strain, on the other, very marked water strain. It has been found that intermediate degrees of water strain are associated with intermediate degrees of closure. Data demonstrating this effect are given in Fig. 2 which shows the relationship between relative turgidity and stomatal aperture in the upper epidermis for cotton at Namulonge and at Ukiriguru. Only values from the periods 11 a.m.–3 p.m. inclusive are given for the following reasons: (*a*) Even under drought conditions relative turgidity is generally highest between 7 and 9 a.m., at a time when stomatal aperture tends to be low owing to hour of day and solar radiation effects (see below). (*b*) From 4 p.m. onwards the effects of low relative turgidity would be reinforced by the closing effects of hour of day and solar radiation. By considering only values for the middle of the day the effects of hour of day and solar radiation are minimized.

The smallest stomatal apertures were recorded for values of relative turgidity of about 70 per cent., and consistently large values were obtained when the relative turgidity was 85 per cent. or more. Similar results were obtained in studies on the stomata in the lower epidermis. There was no evidence to suggest different relationships between stomatal aperture and relative turgidity for cotton grown at the two centres. Some results obtained at Serere Experiment Station in Uganda suggest that a similar response to low turgidity may be shown by cotton there.

That the stomatal closure associated with water strain is not a permanent injury effect was shown at Namulonge during a period of water strain in September and October 1957, when rain-grown cotton plants developed very few new leaves, and stomata in such leaves as were held regularly showed complete closure by 3 p.m.; this was associated with values of relative turgidity of less than 80 per cent. However, small showers of rain were sufficient to restore leaf turgor and allow limited stomatal opening. Heavy rain at the end of October was followed by a period in which full stomatal aperture was regularly obtained, and was also associated with high values of relative turgidity. Irrigated cotton grown in adjacent plots did not show appreciable stomatal closure by 3 p.m. at any stage during the period. Data showing these points are presented in Fig. 3.

Further data on the relationships between stomatal aperture and hour of day, solar radiation, temperature, and relative turgidity for leaves with relative turgidity greater than 85 per cent. are shown in Fig. 4. In consideration of these results the hour of day at which maximum stomatal aperture should occur ($t_{\max}$) was computed from data for both leaf surfaces. The hour-of-day data was then coded so that the opening and closing cycles of stomatal activity were superimposed on an hour scale with $t_{\max} =$ hour 0. Thus, after coding, the relationship between hour and aperture was negative. The assumed linear relationship between stomatal aperture and each of the other variables was then calculated. The correlations are presented in Table 2.

Stomatal aperture is highly significantly correlated with hour of day and solar radiation, and also, for stomata in the upper epidermis, with temperature. The correlations with relative turgidity are not significant, and in the subsequent analyses this factor was omitted. It will be noted that the relationship between hour of day and solar radiation and temperature (both constant

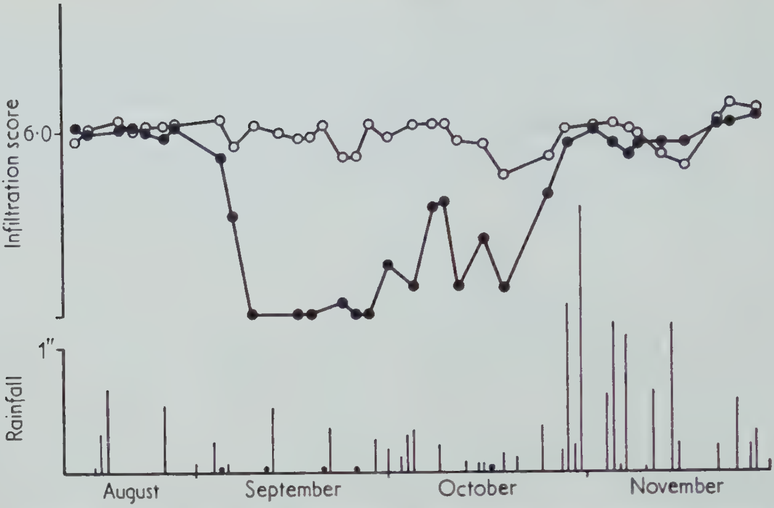

FIG. 3. Stomatal aperture at 3 p.m. for irrigated (open circles) and rain-grown cotton (closed circles) at Namulonge during the 1957–8 season. Rainfall data are also given.

TABLE 2

The Inter-correlations between Stomatal Aperture, Hour of Day, Solar Radiation, Temperature, and Relative Turgidity. Data for the Upper Epidermis are given on the Right-hand side of the Diagonal, Data for the Lower Epidermis on the Left

	Stomatal aperture	Hour of day	Solar radiation	Temperature	Relative turgidity
Stomatal aperture . .	—	−0·77	0·77	0·59	0·22
Hour of day . . .	−0·77	—	−0·71	−0·53	0·29
Solar radiation . .	0·68	−0·81	—	0·50	0·15
Temperature . . .	0·30	−0·31	0·50	—	−0·45
Relative turgidity . .	−0·08	−0·05	−0·15	−0·45	—

Upper epidermis: $t_{\max}$ = 1.12 p.m.
Lower epidermis: $t_{\max}$ = 12.20 p.m. } local time.

Values of r for n = 50

P	0·05	0·01	0·001
r	0·273	0·354	0·443

factors) differed for upper and lower surfaces. This anomalous situation arose because of differences in values of $t_{\max}$ for the two surfaces. Since it was not certain that the differences in $t_{\max}$ were valid, it was decided to take the mean of the two values (12.46 p.m.) as a value of $t_{\max}$ common for both sets of stomata. Accordingly multiple correlation analyses were made on the data using the calculated values of $t_{\max}$ and also the mean value. Values for the standard partial correlation coefficients from these analyses are given in Table 3. It will be seen that the hour-of-day, solar radiation, and temperature factors together account for at least 60 per cent. of the variation in the observed values of stomatal aperture.

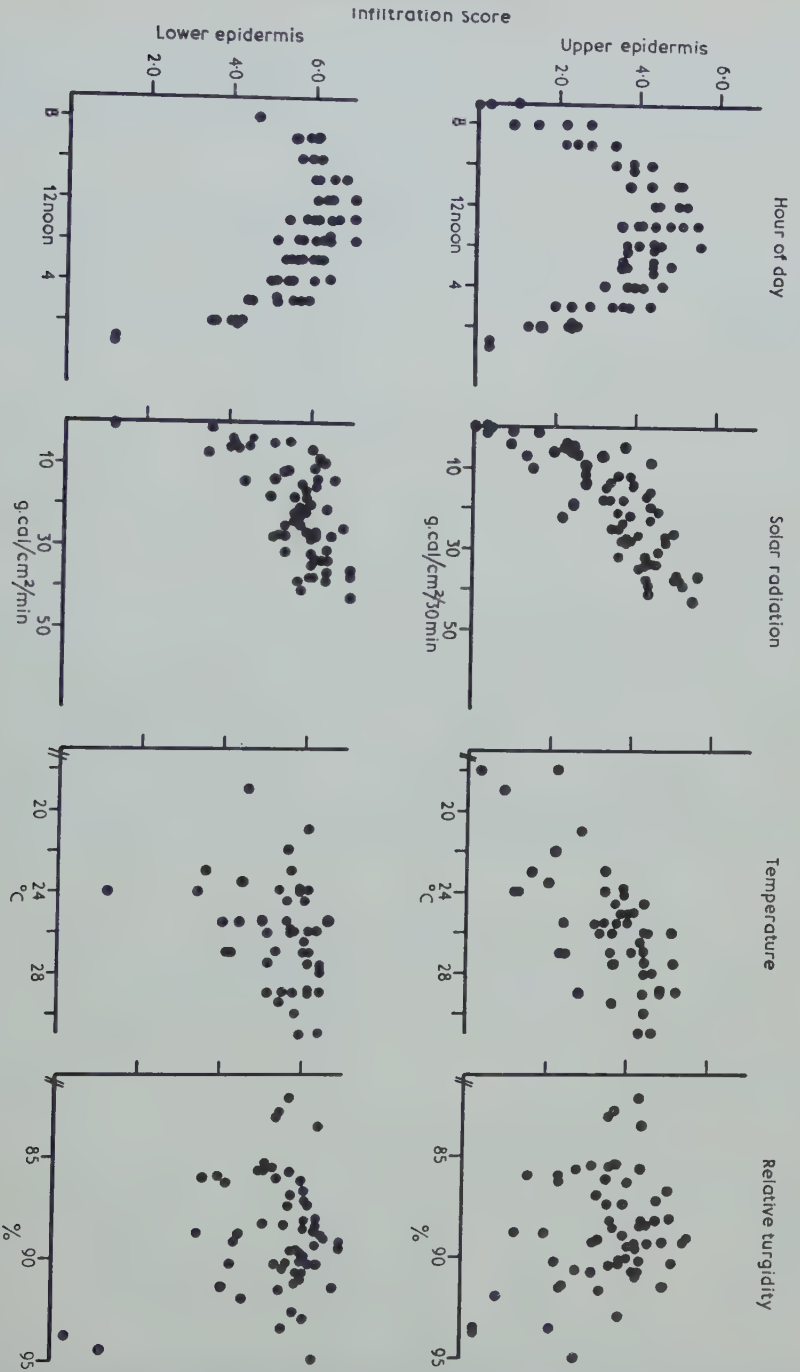

FIG. 4. Scatter diagrams showing the relationships between hour of day, solar radiation, temperature, and relative turgidity, with infiltration score for stomata in the upper and lower epidermis.

TABLE 3

The Standard Partial Correlation Coefficients between Stomatal Aperture, and Hour of Day, Solar Radiation, and Temperature. The Figures in Parentheses Represent the Percentage of the Total Variation in Aperture Accounted for by each Factor

	Upper epidermis		Lower epidermis	
t_{max}	1.12 p.m.		12.20 p.m.	
Hour of day	−0·396	(29·4)	−0·633	(46·8)
Solar radiation	0·390	(28·9)	0·158	(11·7)
Temperature	0·183	(13·6)	0·020	(1·5)
R^2	—	0·719	—	0·600
t_{max}	12.46 p.m.		12.46 p.m.	
Hour of day	−0·432	(31·5)	−0·521	(34·9)
Solar radiation	0·309	(22·4)	0·321	(21.6)
Temperature	0·248	(18·1)	−0·090	(6·1)
R^2	—	0·720	—	0·626

The hour-of-day factor accounts for most variation whilst temperature accounts for only 18 per cent. at the best.

The estimates of temperature used in the analyses refer to those in a standard screen held adjacent to the plants under study. It was thought that leaf temperatures, and especially those of insolated leaves, might be rather higher than the screen temperatures, and a series of investigations were made into this question using thermocouples either threaded though the leaves, or else gently held on to the lower surface of the leaf with small wooden clips. The couple junction was never exposed to direct solar radiation. Results from a series of determinations on exposed leaves on the outside of the plants, and on shaded leaves towards the base of the plants, are given in Table 4.

TABLE 4

Estimates of Ambient and Leaf Temperatures (°C.) on 7.10.58. Each Value is the Mean from Four Observations. Some data for I are also given

Time	Ambient	Exposed leaves	Shade leaves	*I*
9.30– 9.40 a.m.	21·0	24·4	21·2	5·6
9.40– 9.45	20·8	23·8	20·8	—
9.50– 9.55	20·8	22·2	20·8	—
10.00–10.05	20·6	24·2	21·0	5·6
(Cloudy conditions, occasional direct sun.)				
3.00–3.05 p.m.	26·1	28·8	26·5	5·4
3.09–3.14	26·0	30·8	26·4	—
3.15–3.20	25.8	30·4	26.0	—
3.24–3.29	25.8	30.8	26.0	—
3.33–3.38	26.4	31.6	26.8	5.2
(Specks of cloud, much direct sun.)				

Ambient temperature was estimated using a thermocouple held in dense shade within the plants, and the fluctuations observed are probably associated with differences in wind speed. It will be noted that the temperature of shaded leaves was close to that of the ambient, but that exposed leaves showed temperatures considerably higher than the ambient. The data suggest that the temperature factor in relation to stomatal aperture may be under-estimated by considering temperatures within a screen.

DISCUSSION

The pattern of stomatal behaviour for cotton under East African conditions appears to be similar in most respects to that described by Weindling (1948) for cotton grown in Texas. However, Weindling states '. . . closing of stomata is less regular than opening, particularly with stomata on the upper surface'. No evidence to support this was found in the present study. When the hour-of-day data for turgid leaves were examined, the slopes indicating the rates of the opening and closing movements were found to be similar. From the data in Fig. 3 there is no reason to suppose that the behaviour of stomata in the upper surface of the leaf is less regular than that of those in the lower.

That water strain is associated with stomatal closure in cotton has long been known (Balls, 1912; Lloyd, 1913). However, the inter-relationships between relative turgidity, and solar radiation, hour of day, and temperature have not previously been assessed. From the present results the closing effects of water strain must be seen as tending to over-ride the opening effects due to hour of day and solar radiation. The possibility of an interaction between temperature and relative turgidity whereby closure at low values of turgidity is accelerated at high temperatures cannot be disregarded, especially since these two factors are negatively correlated (Table 2).

The mechanism whereby water strain causes stomatal closure is not completely understood. Heath and Meidner (1958) found that the intercellular-space carbon-dioxide concentration increased for wheat leaves suffering water strain, and suggested that this might contribute to the observed stomatal closure. A similar mechanism may operate in cotton.

The effect of changes in water status of epidermal cells on stomatal movement has been considered by Milthorpe and Spencer (1957). They found that with a rapid loss of leaf water, stomatal opening occurred. This was followed, on restoration of the water-supply, by a stomatal closure; it was concluded that this effect could be caused by a disturbance of the equilibrium in water contents between the epidermis and the mesophyll (Williams, 1950). Unpublished experiments have shown a similar behaviour of stomata in isolated leaves of cotton. However, for plants in the field, stomatal opening associated with water strain has never been observed, and this would support Milthorpe's and Spencer's suggestion that the opening does not occur when changes in leaf-water content are gradual.

Certain shortcomings of the multiple correlation analysis should be pointed out. Firstly, it is clear from Table 2 that solar radiation is closely correlated with hour of day, and, to a lesser extent, with temperature. It may be argued that lack of independence between these parameters vitiates the analysis. However, omission of data for either solar radiation, or hour of day, considerably reduces the amount of variation in stomatal aperture accounted for. Also the partial correlation coefficients (Table 3) show that all three factors, considered as they are, independently of each other, have effects of different magnitude on stomatal aperture.

Evidence to suggest that the hour-of-day effect may be a true diurnal rhythm, probably similar to that observed in *Rumex* by Sayre (1926), and in wheat by Heath and Russell (1954), was obtained from an unpublished experiment in which stomatal resistance was estimated using a Wheatstone Bridge porometer for an isolated leaf kept in continuous darkness. Gradual opening of stomata was observed between 8 a.m. and 12 noon followed by closure complete by 6 p.m. This effect was also present, though much less pronounced, during the second day in darkness. The mechanism involved in these movements is unknown.

A second shortcoming of the multiple correlation analysis is that the relationships between stomatal aperture, hour of day, and solar radiation are assumed to be linear. This is not so. In particular, for stomatal aperture in the lower surface (Fig. 3) a linear relationship with solar radiation would give a value for I of about 4·0 for a zero value of radiation, whereas in darkness the observed value for I is zero. Dale (1959) fitted logarithmic and quadratic curves to data for solar radiation and stomatal aperture for the upper epidermis; these curves had correlation coefficients of 0·79 and 0·83 respectively. There was no indication of a stomatal closure in cotton with solar radiation intensities of up to 1·4 g.cal./cm.^{2}/min. although it may be noted that increases in solar radiation above about 1·0 g.cal./cm.^{2}/ min. do not have a large effect in increasing stomatal aperture. These results stand in contrast to Nutman's (1938) claim that high radiation intensities were associated with stomatal closure in *Coffea arabica*. If it were possible to consider a quadratic relationship between I and solar radiation in the multiple correlation analysis a greater proportion of the variation in stomatal aperture might be accounted for.

Light intensity as such has not been measured in these observations, and the relationship between light intensity and solar radiation is not known. It seems probable that it is the light-energy fraction of solar radiation that is responsible for the opening effect, firstly because of the known effect of light on stomatal aperture (e.g. Heath and Russell, 1954), and secondly because of the comparatively minor effect of temperature on stomatal behaviour.

The larger effect of temperature on stomata in the upper epidermis may be a real one and it is possible that this is associated with a higher temperature at this leaf surface. This seems a reasonable hypothesis since the leaves used in this study were usually exposed to direct radiation. However, comparison of leaf temperatures at the two surfaces under field conditions is difficult since thermo-couples will accept heat if exposed to direct radiation. When the leaf was shaded artificially, thus altering the experimental conditions, no difference in temperatures at the two surfaces could be detected.

Wilson (1948) found that stomatal aperture of cotton, estimated porometrically using a fixed cup, increased with temperature up to 30° C., but with higher temperatures a slight closure occurred. Since Wilson did not estimate leaf-water status it is uncertain whether this closure was due to temperature alone or to water strain. From Table 2 it is apparent that there is a significant negative correlation between temperature and relative turgidity.

The effect of carbon-dioxide concentration in controlling stomatal aperture is now well known and the effects of temperature on intercellular-space carbon-dioxide concentration have been investigated (Heath and Orchard, 1957; Heath and Meidner, 1957, 1958). Meidner and Heath (1959) showed for onion leaves swept with carbon-dioxide-free air that the stomatal response was to open with increasing temperature, in contrast to the closure observed at temperatures above 30° C. when the intercellular-space carbon-dioxide concentration was allowed to rise. With the high levels of solar radiation observed in the present study, concurrent photosynthesis in cotton leaves may well keep the intercellular-space carbon-dioxide concentration at a low level, thus indirectly allowing a large stomatal aperture at high temperatures.

ACKNOWLEDGEMENTS

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Take-off of Mould Spores in Relation to Wind Speed and Humidity

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With six Figures in the Text

ABSTRACT

Using horizontal tube-cultures of various moulds belonging to the genera *Thamnidium*, *Phymatotrichum*, *Trichothecium*, *Piptocephalis*, *Trichoderma*, *Mucor*, and *Mycogone*, the take-off of dispersal units (usually spores) under the influence of air currents of various speeds and of different humidities has been studied. It is found that in all the dry-spore forms the number of spores set free increases as the speed of the air stream rises. Further, at any given air-stream rate, the numbers of spores set free are greatest in the first interval of time and rapidly fall off in subsequent intervals. In all species, spores are more readily set free in air streams of relatively low as compared with those of relatively high humidity. Although *Trichoderma viride* has been regarded as a slime-spore fungus, its conidia can readily be blown from the conidiophores. No spores could be induced to take off from *Mucor ramannianus* even at the highest air speeds used.

INTRODUCTION AND METHOD

ALTHOUGH the violent discharge of fungal spores has been the subject of considerable investigation, little is known with any precision about the factors involved in the liberation of those that are not actively shed. This work was undertaken to study the take-off of spores or more complex propagules from conidiophores and sporangiophores under the action of air currents of differing velocity and humidity.

The fungi used were *Thamnidium elegans* Link, *Phymatotrichum fimicola* Dring, *Trichothecium roseum* Link ex Fries, *Piptocephalis virginiana* Leadbeater and Mercer, *Trichoderma viride* Pers. ex Fries, *Mucor ramannianus* Moller, and *Mycogone perniciosa* Magn.

The procedure adopted was to grow the moulds under investigation in horizontal glass tubes, to draw through the tubes air of known humidity and of definite speed, and to impact the liberated spores on the sticky slides enclosed in an impactor unit.

The glass tubes used for culturing the fungi were 20 cm. in length with an internal diameter of 1·5 cm. In filling the tubes with nutrient medium each was stoppered at one end with a rubber bung and kept in a vertical position. The hot unset nutrient agar was poured in until it reached exactly half-way up the tube. These tubes were then autoclaved for 15 minutes at 20 lb. pressure. Immediately after taking the tubes out of the autoclave, the open

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ends were also stoppered with sterilized bungs. Each tube was then placed horizontally to cool. The fact that it had been filled exactly to the half-way mark ensured that when the agar set it occupied half the area of the cross-section of the tube.

When the medium had set, the tubes were brought into the inoculating room. The rubber bungs were removed, the end portions of the agar strip in the tube were cut with a special sterilized tool to give a clean edge, and both ends of the tube were plugged with sterilized cotton-wool. Care was taken to ensure that the horizontal length of the agar strip, after cutting the edges, was equal in each tube.

For inoculation one plug of the prepared tube was removed and the whole surface of the medium inoculated lengthwise with the desired fungus, using a long sterilized looped nichrome wire. The tube was then plugged again.

Special horizontal wooden racks were used for holding the tubes during the growth of the cultures, which were left undisturbed at a constant temperature (20° C.) and illuminated with 'day-light' fluorescent tubes giving an intensity of 500–1,000 lux.

When cultures were freely sporulating, a tube was ready for use in an experiment.

The apparatus used consisted of the following units serially connected: a high-vacuum pump, a flow meter (rotameter), an impactor unit, the horizontal tube containing the mould culture, and either a humidifier or two drying towers.

The air was sucked by the pump through the humidifier. The air speed was controlled by a screw clip. In most experiments this was placed between the rotameter and the impactor unit. This led to incorrect readings of the rotameter and subsequent recalculations of air speed had to be made. In later experiments the clip was placed in its proper position between the pump and the rotameter. The humidifier consisted of a large water reservoir (10 litres capacity) and a system of 14 glass inlet tubes ($0{\cdot}4 \times 42{\cdot}0$ cm.) with a wider outlet tube of 1·6 cm. diameter. These tubes were inserted into the reservoir through a rubber stopper.

In order to make sure that a humid air stream was free from water droplets, it was passed through a large empty jar, before circulating over the fungal growth.

To obtain a current of very low humidity the air was sucked through calcium chloride towers.

The humidity was determined with the aid of wet-and-dry-bulb thermometers placed in the air stream.

The rotameter recorded rates in litres per minute. However, rates of the air stream have been recalculated in terms of metres per second, on the assumption that air is flowing through half the cross-sectional area of the culture-tube. This is subject, however, to a certain error as the aerial growth of the fungus slightly reduces this cross-sectional area. Again, it is to be noted that the calculated rates of flow are average rates for the tubes as a whole.

Clearly the flow will be slower, due to frictional drag, in immediate contact with the wall of the tube and with the surface of the culture, and faster in the free central region.

Liberated spores were trapped in the impactor, which consisted of the first unit of a 4-jet 'cascade impactor'. Connexion between the entry orifice of the impactor and the culture-tube was made with a specially constructed steel fitting and rubber tubing (Fig. 1).

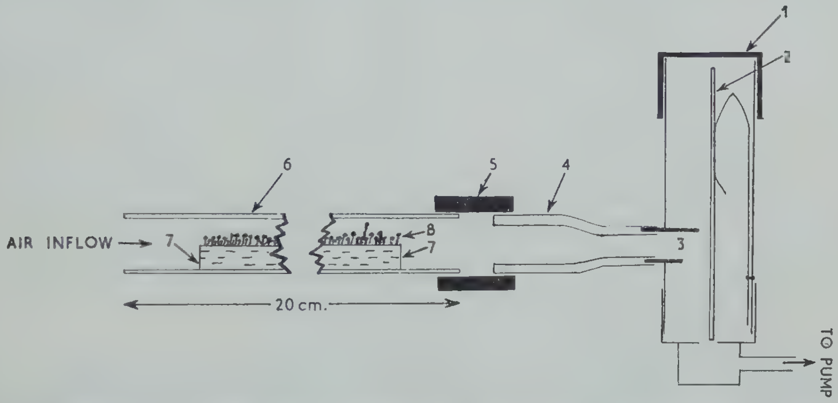

FIG. 1. Diagram of impactor unit connected to a culture-tube (half real size): 1, moulded rubber cap; 2, glass slide; 3, entry slit; 4, steel connector; 5, rubber tube; 6, culture-tube (middle part omitted); 7, agar strip; 8, aerial growth of fungus.

A standard microscope slide, smeared evenly with a thin film of vaseline, was put into the impactor and the liberated spores were deposited on it, often forming a visible trace. Exposed slides were stored for subsequent counting.

The density of the spore deposit varied to some extent in different parts of the trace (15 mm. × 3 mm.) and it was necessary to adopt a standard method of sampling. This was done by making three equally spaced traverses at right angles to the trace and along each of these the spores in 15 high-power fields, at regular intervals, were counted. In all cases the same traverses were made, the positions being located by the verniers of a mechanical stage. In the graphs and tables the figures given represent the total number of propagules counted in the 45 high-power fields.

THAMNIDIUM ELEGANS

This fungus has large terminal sporangia and small sporangioles produced on richly branched laterals developed from the main sporangiophores. As in *Mucor* the main sporangium is converted at maturity into a sporangial drop. On the other hand the sporangioles do not become droplets, but break off at the point of their attachment with the sporangiophore as dry structures which are wind dispersed (Ingold, 1940).

Experiments with this fungus showed that air currents up to 5 m./sec., irrespective of humidity, failed to dislodge the spores from the large terminal sporangia, but the sporangioles were easily detached as individual units.

Spore liberation into air streams of different humidity

An experiment was performed to compare the numbers of sporangioles liberated into air streams of constant velocity and of different humidities.

Twenty culture-tubes were prepared and from them twelve were selected by eye for their similarity. Six of these, chosen at random, were subjected to a dry air current (46 per cent. R.H.) and the other six to a damp one (91 per cent. R.H.). The rate of air flow was 5 m./sec. For each culture-tube six successive readings were taken with the humidity and rate of flow kept constant. Each reading involved the passage of air over the culture for one minute, the pump being stopped between the readings. Six separate tubes were employed at the lower, and six at the higher, humidity. The results are shown graphically in Fig. 2A. It will be seen that there is a general tendency for the number of sporangioles caught on the impactor slide to fall off in successive readings. Further, it is clear that the numbers liberated into the dry air are very much greater than those liberated into the humid air.

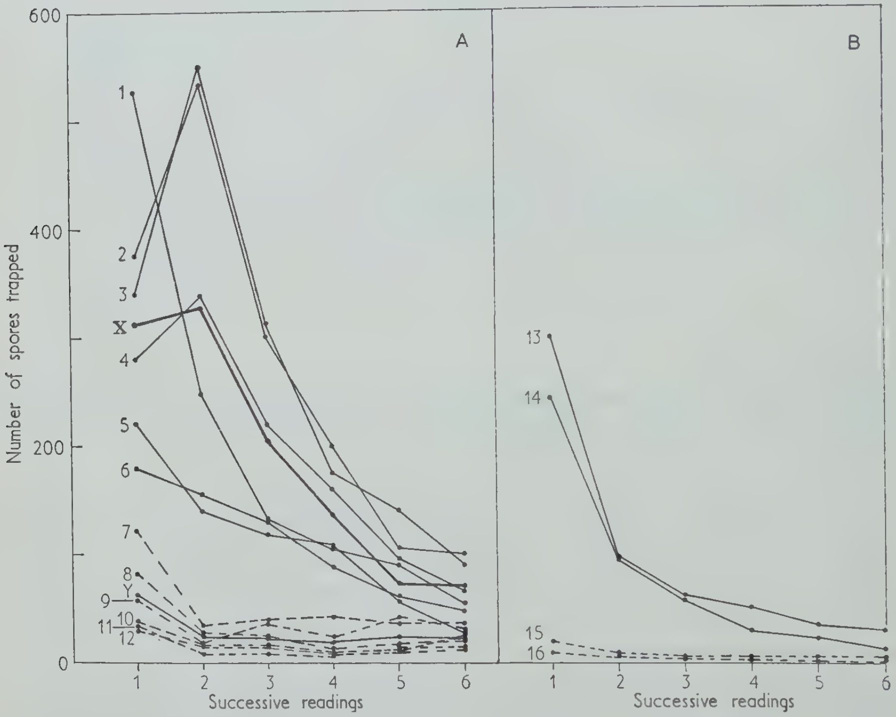

FIG. 2. *Thamnidium elegans*. A. Number of spores caught on impactor slide in six successive one-minute intervals; graphs 1–6 for culture-tubes subjected to dry air stream (46% R.H.), X being the mean curve; graphs 7–12 for tubes subjected to damp air stream (91% R.H.), Y being the mean. Air speed 5 m./sec.

B. A similar experiment, graphs 13 and 14 showing catch from tubes in dry air stream (27% R.H.) and graphs 15 and 16 in damp stream (96% R.H.).

In a further similar experiment in which there was a much greater difference

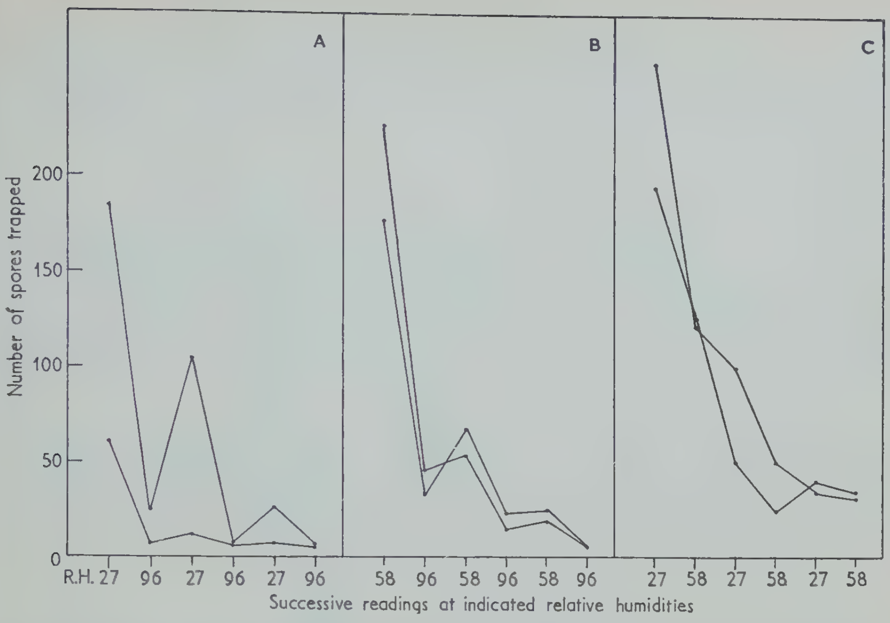

FIG. 3. *Thamnidium elegans.* Number of spores caught on impactor slide in successive one-minute intervals of time with alternation in the humidity of the air stream as indicated and with different contrasted humidities in the three separate experiments, A, B, and C. Air speed 5 m./sec.

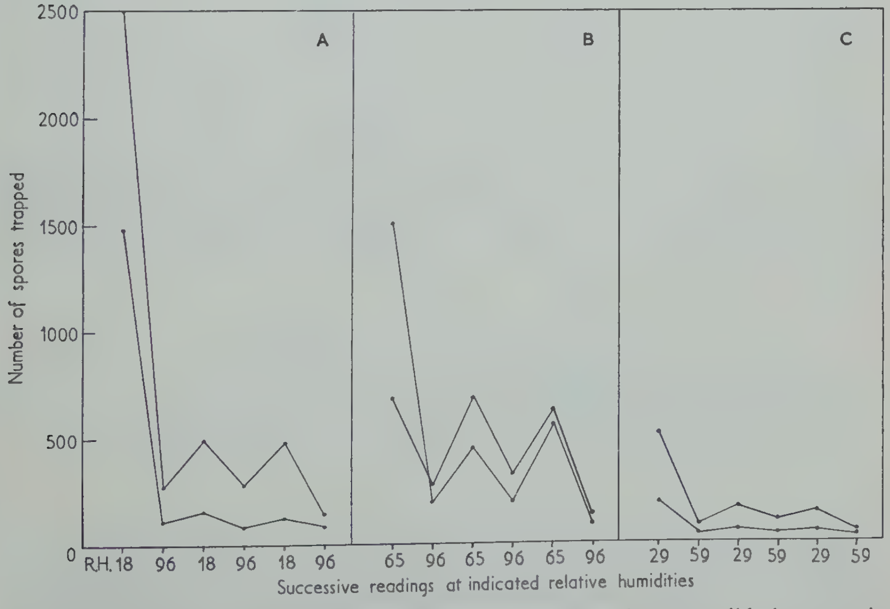

FIG. 4. *Phymatotrichum fimicola.* Number of spores caught on impactor slide in successive one-minute intervals of time with alternation in the humidity of the air stream as indicated and with different contrasted humidities in the three separate experiments. Air speed 5 m./sec.

in the two humidities used (27 per cent. R.H. and 96 per cent. R.H.) the same general picture emerged, except that in the almost saturated air hardly any sporangioles were blown off at all (Fig. 2B).

Liberation of sporangioles under alternate dry and humid air currents

Experiments were performed to compare, in a single tube-culture, the number of sporangioles liberated into air streams of two different humidities, a dry air current being followed by a damp one and then by a dry and so on. For each pair of humidities two separate experiments were carried out. In the three pairs of experiments the two contrasting humidities used differed considerably.

The results are shown graphically in Fig. 3. It is clear that, where one of the two air streams is very dry and the other very damp (Fig. 3A), change from damp to dry air always leads to an increase in sporangiole liberation. However, where the two humidities are not so contrasted and both of them are fairly low, this phenomenon is not clearly shown (Fig. 3C).

PHYMATOTRICHUM FIMICOLA

The conidia of this fungus, recently described by Dring (1959), are produced in dry spherical heads on erect conidiophores up to 1 cm. long. In the head itself the spherical spores are borne on sterigmata arising from dichotomous lateral branches crowded in the apical region of the conidiophore.

When air currents were sucked through the horizontal culture-tubes containing this species, spores were readily freed as individuals. The detachment of whole branchlets as described by Dring (1959) was not observed.

The results obtained by drawing alternately currents of two contrasted humidities through the same culture-tube are shown in Fig. 4.

There is clearly a tendency for a decrease in the rate of spore liberation when air humidity is increased, superimposed upon the general tendency for decreased liberation in successive intervals. It is to be noted that rather low rates of liberation indicated in Fig. 4C are due to poorly sporulating cultures in these particular experiments.

TRICHOTHECIUM ROSEUM

A recent account of the morphology of the conidial apparatus of this common and well-known fungus has been given by Ingold (1956).

The conidiophore is simple (100–250 μ long) and bears at its apex a staggered chain of often 20 or even 30 spores.

Liberation of the spores in relation to the rate of flow of the air current

For this experiment 50 culture-tubes were prepared and from them 36 were selected by eye as being fairly uniform. These were then sorted in a random manner into six groups each of six tubes. In each group the tubes were subjected to air streams of constant humidity and speed but, although the humidity did not vary, the speed varied from one group to another. For

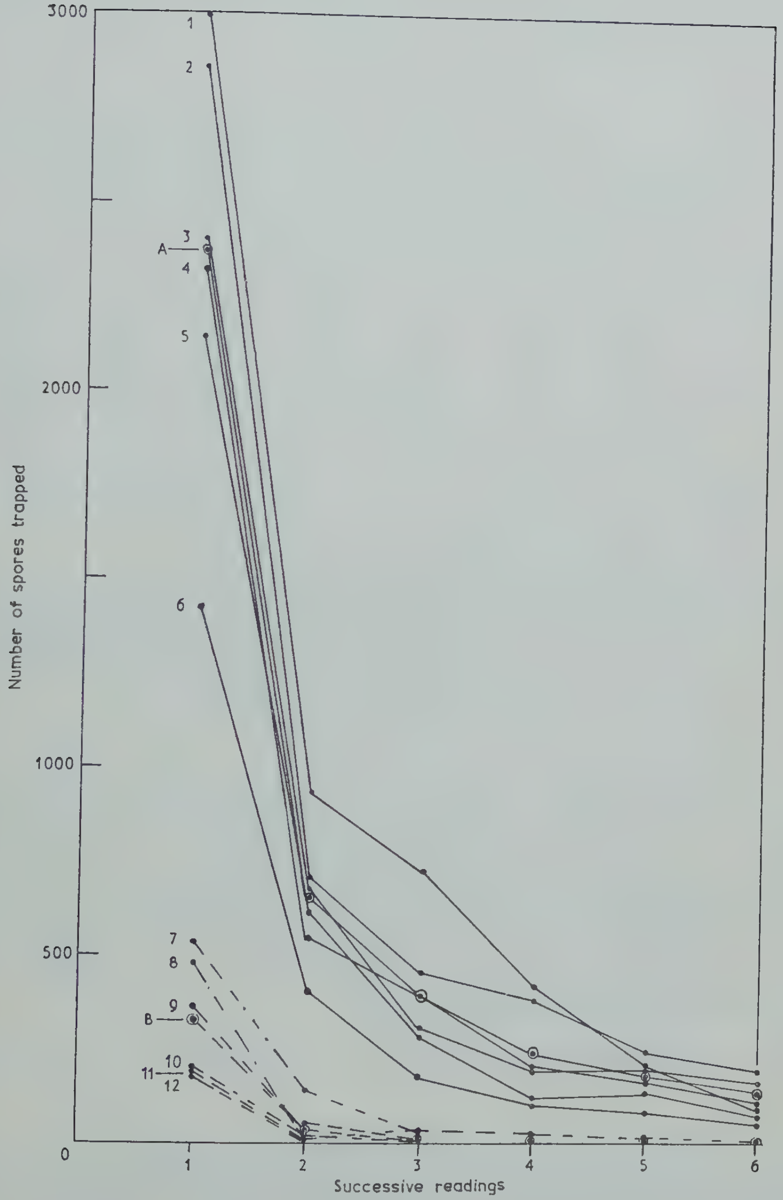

FIG. 5. *Trichothecium roseum.* Number of spores caught on impactor slide in six successive one-minute intervals; graphs 1–6 for culture-tubes subjected to dry air stream (50·5 % R.H.), A being the mean curve; graphs 7–12 for the tubes subjected to damp air stream (95 % R.H.), B being the mean. Air speed 5 m./sec.

each tube only one determination was made, namely the catch in the impactor following the passage of the air stream for a period which varied from 75 to 200 seconds but in all cases involved the flow over the culture of the same volume of air.

TABLE 1

Catch of Spores in the Air Currents of Different Speeds at Constant Humidity (R.H. 31%). Thirty-six Separate Culture-tubes Used

Speed (m./sec.)	Catch of spores from individual tubes						Mean
	1	2	3	4	5	6	
1·7	53	133	76	182	162	184	132
2·5	344	312	363	464	452	478	399
3·3	855	669	811	1,415	2,757	953	1,234
5·0	2,711	1,431	4,305	5,218	4,881	1,700	2,358
6·7	4,215	4,794	8,161	6,036	6,577	5,488	5,879
10·0	8,543	9,596	12,955	13,296	16,745	13,114	12,375

The results (Table 1) show that the rate of spore liberation increases with the increase of the wind velocity.

The readings obtained with a constant air speed (say 1·7 m./sec.) varied from tube to tube, this probably being due to lack of uniformity in the sporulation in the tubes, in spite of similarity to the eye. However, it is clear that increased speed of the air stream passing over the fungus culture resulted in a great increase in spore liberation; an increase of 0·8 m./sec. in air speed produced an effect far exceeding any variation between tubes at the lower speeds.

Catch of spores in successive intervals of time at contrasted humidities

To investigate spore liberation in successive intervals of time, the procedure adopted was similar to that used for *Thamnidium elegans*. The results obtained are given in Fig. 5. It is clear that the number of spores blown off under constant conditions of air flow and humidity falls off in successive intervals of time. Further, the results again show that the rate of spore liberation in relatively dry air (50·5 per cent. R.H.) is much higher than the rate in more humid air (95 per cent. R.H.).

Catch of spores in successive intervals with alternation of dry and humid air streams

A single culture-tube was subjected alternately to air streams of two contrasted humidities. The treatment with each humidity lasted for one minute.

Three different experiments involving different pairs of contrasted humidities were performed and the results obtained are shown in Fig. 6.

The successive readings did not give a gradually falling curve as did the readings obtained with constant humidity in the previous experiment, but in each case the graphs show an alternate rise and fall according to whether the humidity of the air stream is relatively high or relatively low.

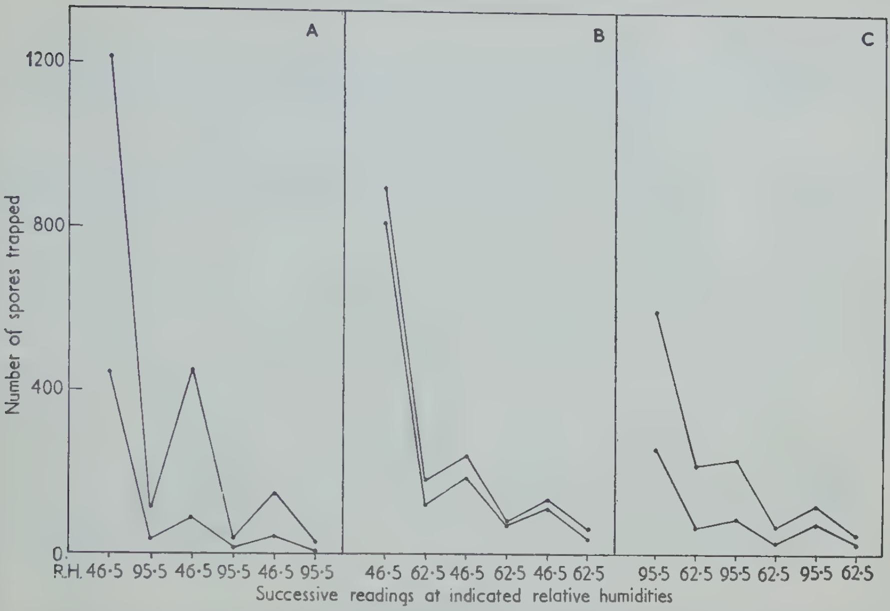

FIG. 6. *Trichothecium roseum.* Number of spores caught on impactor slide in successive one-minute intervals of time with alternation in the humidity of the air stream as indicated and with different contrasted humidities in the three separate experiments A, B, and C. Air speed 5 m./sec.

PIPTOCEPHALIS VIRGINIANA

The morphology of this fungus has been described by Leadbeater and Mercer (1957). It is a parasitic mucoraceous fungus, grown in the culture-tubes on *Mucor circinelloides*. When sporulation occurs little of the host is to be seen.

The spores of this fungus were not liberated as individual units. They were found deposited on the impactor slide in the form of 'spore-rods', each with 3–8 cylindrical spores, and very frequently these 'rods' were still joined to their head-cell.

Ten culture-tubes were prepared for this experiment, five being subjected to air streams of 53·5 per cent. and five to streams of 90 per cent. relative humidity. Six successive readings were obtained from each tube. Each reading involved an air stream of constant speed 4·6 m./sec. flowing for a period of one minute.

The results obtained are shown in Table 2. Clearly the number of spores liberated into the air stream in the first period was much higher than in subsequent periods. Further, it will also be noticed that the later readings, where values were low (Table 2), varied little as between relatively dry and relatively humid air. This is probably due to the fact that in the drier air nearly all spores were blown off with the first blast of the air stream and there were not many left for liberation in the following periods. On the other hand in the more humid air streams the initial liberation of spores was

TABLE 2

Catch of Spore-Rods from Two Sets of Tubes at Two Different Humidities. Consecutive one-minute readings in each case (age of culture—9 days; rate of flow—3·6 m./sec.)

R.H. and temp.	Tube number	Catch of spore-rods in successive 1-minute intervals					
		1	2	3	4	5	6
53·5% at 15·5° C.	1	1,152	161	39	35	30	24
	2	1,444	87	21	5	4	16
	3	1,257	78	55	17	15	5
	4	4,429	172	51	27	14	2
	5	2,821	106	21	20	9	6
Average		2,221	120	37	21	14	11
90% at 16° C.	6	408	80	38	28	24	21
	7	276	99	86	60	41	26
	8	217	72	36	17	21	0
	9	155	62	17	12	6	5
	10	660	137	26	10	3	10
Average		343	90	41	25	19	12

TABLE 3

Catch of Spores in Two Sets of Tubes at Two Different Humidities. Consecutive one-minute readings in each case (age of culture—6 days; rate of flow—8·8 m./sec.)

R.H. and temp.	Tube number	Catch of spores in successive intervals of time					
		1	2	3	4	5	6
45·5% at 20·5° C.	1	31,080	2,236	2,164	714	644	724
	2	16,930	624	642	514	434	225
	3	8,446	2,192	903	896	442	136
	4	7,579	3,197	1,077	1,938	1,347	666
	5	5,702	1,206	557	327	307	297
	6	5,301	1,049	2,439	912	269	267
Average		12,506	1,751	1,297	884	574	386
91% at 20° C.	7	1,182	413	1,381	423	60	38
	8	774	243	112	1,029	135	189
	9	1,508	727	122	80	35	96
	10	13,223	3,060	1,248	956	812	645
	11	468	49	15	0	0	0
	12	1,348	22	41	0	0	0
Average		3,084	736	477	415	174	161

lower, and relatively more were left behind to be set free in subsequent periods.

TRICHODERMA VIRIDE

Low-velocity air currents fail to liberate any spores, but at high speeds these are blown off in abundance, therefore the fungus cannot be considered entirely a slime-spore type. It is, in part at least, a dry-spore fungus.

When air at sufficient speed was drawn through the tubes containing cultures of *Trichoderma viride*, large numbers of spores were liberated and deposited on the impactor slide either as individual spores or, more frequently, as groups of 20–30 spores.

To study the effect of different levels of humidity on spore liberation, twelve culture-tubes were selected as being reasonably similar. Through six of these dry air (45·5 per cent. R.H.) was passed, and through the other six damp air (91 per cent. R.H.), the air speed in all cases being 8·8 m./sec. For each tube six successive one-minute readings were made (Table 3).

It will be noticed that damp and dry air currents do not produce such striking differences in spore liberation as were produced in similar experiments with *Thamnidium elegans* and *Trichothecium roseum*. Sometimes, even, the number of spores caught from a particular tube in humid air was greater than the number caught from a tube in drier air. However, the mean values of the spores caught from two sets of tubes at the two humidities show a clear and consistent difference. It will also be noticed that the successive readings obtained from a single tube, in constant humidity, do not always show continually falling values.

MYCOGONE PERNICIOSA

Out of the ten tubes prepared for the experiment with this fungus, three, selected at random, were subjected to air streams of 55 per cent. R.H. and three to air of 91 per cent. R.H. The speed of the air (8·3 m./sec.) was kept constant. Each reading involved a period of flow for 30 seconds. Results are shown in Table 4. Clearly the numbers of spores liberated into the damp stream in the first period are much higher than in subsequent periods. In drier air there is not such a striking difference between liberation in the first and in subsequent periods, but the numbers fall off gradually. However, the general feature, noted in the other dry-spore moulds, that moving dry air detached many more spores than damp air, is clearly shown.

TABLE 4

Catch of Spores in Two Sets of Tubes at Two Different Humidities. Consecutive 30-second readings (age of culture—7 days; rate of flow—8·3 m./sec.)

R.H. and temp.	Tube number	Catch of spores in successive 30-second intervals					
		1	2	3	4	5	6
55% at 19° C.	1	7,119	1,130	682	540	295	206
	2	10,983	2,130	947	615	542	395
	3	8,046	1,101	592	361	241	235
Mean		8,716	1,454	744	505	349	278
91% at 19° C.	4	5,132	457	248	89	97	19
	5	2,771	173	66	45	27	14
	6	3,076	125	73	33	19	22
Mean		3,660	252	129	56	48	18

MUCOR RAMANNIANUS

This is a common soil fungus. The sporangiophores are mostly unbranched and produce sporangia with diffluent walls.

To investigate the effects of air currents on spore liberation, culture-tubes of this species were subjected to air currents of different speeds and humidities.

The experiments showed that, with air streams of up to 16·6 m./sec. with relative humidity in the range 20–90 per cent., no spores were found deposited on the impactor slides. The pump available did not allow higher speeds to be achieved.

It thus appears that, unlike *Trichoderma viride*, this fungus is entirely a slime-spore type and no spores can be blown off the sporangiophore.

GENERAL RESULTS

The observations presented in this paper show that in *Mucor ramannianus*, a fungus producing a spore-drop, no spores could be detached at the maximum air speed used, but that in *Trichoderma viride*, also regarded as a slime-spore type, spore liberation does occur into air streams which are sufficiently rapid. All the other fungi examined were recognized dry-spore types.

When the conidiophores of *Trichothecium roseum*, *Phymatotrichum fimicola*, *Mycogone perniciosa*, and also of *Trichoderma viride* were subjected to air streams of contrasted humidities, spore liberation into relatively dry air took place more freely than into damp air. This was also true of the sporangioles of *Thamnidium elegans* and of the spore-rods (usually attached to head-cells) of *Piptocephalis virginiana*. It would seem that a general principle is involved.

An experiment to investigate the effect of wind speed on spore liberation in *Trichothecium roseum* showed clearly that the liberation of spores increases with increased velocity.

Further, in all the moulds described in which spore detachment into air currents occurred, the rate of liberation fell off with time, probably due to the exhaustion of the spore supply in the culture.

ACKNOWLEDGEMENT

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Correlation of Tissues in Leaves

1. Absolute Vein-islet Numbers and Absolute Veinlet Termination Numbers

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ABSTRACT

Vein-islet numbers and veinlet termination numbers of leaves were found to be inversely proportional to the area of the lamina. The constants obtained by the multiplication of these values are termed the absolute numbers. This value is constant in those leaves where the differentiation of the veins has been completed. This is shown in the developing leaves of five Solanaceous plants.

INTRODUCTION

DURING routine pharmacognostic analysis of medicinal plants it was observed that some values used in quantitative microscopy have unusually high values in younger leaves. It was therefore decided to investigate the effects of the maturity of leaves on the vein concentrations in laminae, and for this purpose leaves in different state of growth were examined in detail.

The early cessation of apical growth is a remarkable feature of foliage leaves. The final size and form are largely determined by extension growth. There are many foliar organs like cotyledons, bud-scales, bracts, appendages of flowers which apparently follow the anatomical pattern of true leaves.

It has been thought that the vein concentration per unit area in the lamina is a constant for a particular species (Levin, 1929; Hall and Melville, 1951, 1954), but in fact the nature of the vein-concentration in the developing lamina has not been investigated decisively. There is some controversy over the time taken by the veins to differentiate completely in leaves. Hayward (1948) observed in the case of the leaves of *Solanum tuberosum*, 'differentiation of the vascular system is not completed until the blade is fully developed'. But according to Eames and MacDanials (1947), the outline and fundamental structure of most leaves are developed while the leaf is yet minute. Later growth consists in large measure of rapid increase in cell-size and in maturation of mesophyll cells and vascular tissues, which takes place during the period of leaf expansion. After the leaf attains the full size the larger vascular bundles usually increase in diameter by secondary growth.

The concentration of veins in a lamina can be estimated by two methods. One was advanced by Levin (1929), where the number of vein-islets per unit area, formed by ultimate ramifications of veins, are counted (vein-islet

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number). In the other method by Hall and Melville (1951, 1954), free vein endings per unit area are counted (veinlet termination number). All these countings are made from a 4 sq. mm. area of the leaf surface and are expressed as a number per sq. mm. For the assessment of vein-islet number and veinlet termination number in any particular leaf it was found by the author that the average of five different readings taken from the central region of the lamina avoiding the margin, mid-rib, and thicker veins, gives fairly consistent estimates.

MATERIALS AND METHODS

In the case of herbaceous plants leaves are produced in quick succession and these may be found in different states of maturity in a single branch. To avoid genetical and many other variations leaves from a single branch of a plant were analysed. For a single reading 4 sq. mm. area is required and 5 such readings are needed from a single leaf, and for this reason only leaves with sufficient leaf area should be selected. Therefore leaf buds and too small leaves just below them could not be selected for counting, yet there remained a few immature leaves in each series.

For the experimental purpose five species of the family Solanaceae were selected. These are: (i) *Nicotiana tabacum* Linn., (ii) *N. plumbaginifolia* Viv., (iii) *Solanum nigrum* Linn., (iv) *S. ferox* Linn., and (v) *Withania somnifera* Dun. All these plants were grown in the Institute in pots from the seeds of identified plants. The plants which were free from metabolic disorders were collected and pressed in a herbarium press, care being taken to avoid crumpling or folding of the leaves and also that no leaf was lacking on the particular branch preserved. These leaves were then serially numbered, the numbering of the leaves beginning with the obviously protruding leaf bud at the end of the branch. Thus the first few leaves in each series are immature ones and the leaves of higher number are mature ones. The samples were taken from the broadest part of a leaf, avoiding margins and main veins.

Removal of trichomes. The presence of trichomes may cause much annoyance in the examining of the leaves. In extreme cases the trichomes completely obliterate the inner tissues. To have an easy and unimpaired view of epidermis and vascular strands it is necessary to remove the trichomes from the leaves. If the epidermal layer is injured in the process, the inner tissues may also rupture. So the trichomes should be removed in such a manner that the epidermal cells, stomata, vascular strands, &c., can be viewed easily even from a whole-mount preparation. After several trials the following method has been evolved by which probably all types of leaves can be freed from trichomes. But the stubby basal cells of stellate trichomes of some *Solanum* species may not be removed by this process. These do not cause much difficulty in the observations.

Dry and flattened leaves are used to remove the trichomes. After the leaves are fully dried and pressed flat, the trichome-bearing surfaces are rubbed over a rough surface like a filter paper or a stretched soft lint or a stretched muslin or a fine sand-paper (No. '00') according to the type of the leaves. The leaf fragment is held over the grinding surface by the finger-tip and is ground evenly with minimum pressure. The movement should be circular and even pressure on all sides is necessary. When both the surfaces are ground in this way, most of the trichomes are removed. The remaining ones can be dislodged with the help of a paper-cone (made up of filter paper) under a dissecting microscope.

If the leaves are thin and have stiff hairs, filter-paper grinding is needed. But if the leaves are thin and the trichomes are soft and tough, soft lint grinding may be helpful. In case of thick leaves with stiff hairs it is better to grind them over a stretched muslin cloth. The woolly and tough trichomes in leaves like *Hyoscyamus muticus* are hard to remove without causing injury to the leaf. In these extreme cases grinding on fine sand-paper (No. '00') may be required. Much care is needed at the time of grinding and frequent examinations of the surfaces are required. If much pressure is applied during grinding, cracked leaf fragments may result. Traces of moisture make it easy to tear off the epidermis.

The areas of the dried leaves were determined by drawing the outlines of the pressed leaves over 'Whatman No. 1' filter paper and cutting along the outlines of the laminae. By comparing the weights of these papers with the weight of another piece of the same material of known area, the probable areas of these leaves were determined.

From each leaf a portion was cut from the centre between midrib and margin and was freed from trichomes by grinding. The cut portions were cleared by boiling them with conc. chloral hydrate soln. (5:2) in a water-bath, washed in water, and placed in conc. HCl to remove the calcium oxalate crystals. After the acid treatment the leaves were washed thoroughly and mounted with 40 per cent. glycerine or they may be mounted after staining the veins lightly with gentian violet.

Palisade ratio was determined according to Wallis and Dewar (1933); vein-islet number was determined according to Levin (1929); and veinlet termination number was determined according to Hall and Melville (1951, 1954).

OBSERVATIONAL

In *Nicotiana tabacum* and *N. plumbaginifolia* large numbers of mature leaves are borne on a single axis and even very young leaves are big enough for analysis. It was found that the leaves borne in the flower panicle were somewhat different in shape. So these leaves were excluded. In the case of *Solanum spp.* and *Withania* the number of leaves in a branch was not high.

For each plant the area of the lamina (A), palisade ratio (P.R.), vein-islet no. (v.i.), veinlet termination number (v.t.) of the leaves were determined. From each leaf five readings in palisade ratio, vein-islet number, and veinlet termination number were taken and the averages were incorporated in the following tables 1–5. It was observed that with the decrease of the

TABLE 1

Numerical Data for Nicotiana tabacum *Linn.*

Leaf no.	Area of lamina in sq. mm. (A)	Palisade ratio (P.R.)	Vein-islet no. (v.i.)	(A)×(v.i.) in thousands (Absolute v.i.)	Veinlet termination no. (v.t.)	(A)×(v.t.) in thousands (Absolute v.t.)
18	34,080	8·00	6·6	224·9	3·3	112·5
17	32,890	8·60	6·2	203·9	3·8	125·0
16	32,670	8·65	6·4	209·1	4·4	143·7
15	30,100	10·65	7·3	219·7	4·8	144·5
14	24,390	11·05	8·5	207·3	4·5	109·8
13	20,240	12·35	10·4	210·5	7·1	143·7
12	19,130	11·00	10·5	200·9	8·6	164·5
11	17,470	8·65	13·7	239·3	9·5	166·0
10	15,050	9·20	14·9	224·2	8·4	126·4
9	12,660	11·10	16·1	203·8	9·1	115·2
*8	8,241	9·45	19·6	161·5	9·6	79·0
*7	6,966	11·20	19·3	134·5	11·9	82·9
*6	3,322	7·95	29·6	98·3	15·3	50·8
*5	2,400	8·30	31·6	75·8	13·7	32·9
*4	1,224	7·20	47·4	58·0	22·6	27·7
*3	424	5·30	79·6	33·8	55·6	23·6
*2	365	5·65	90·8	33·1	44·8	16·4
*1	165	5·05	88·4	14·6	48·2	8·0

TABLE 2

Numerical Data for Nicotiana plumbaginifolia *Viv.*

Leaf no.	(A)	(P.R.)	(v.i.)	(Absolute v.i.) (A)×(v.i.) in thousands	(v.t.)	(Absolute v.t.) (A)×(v.t.) in thousands
11	3,512	3·50	5·6	19·7	4·8	16·9
10	3,968	3·70	4·8	19·0	4·5	17·9
9	2,777	3·65	7·1	19·7	6·7	18·6
8	2,912	3·35	6·5	18·9	7·2	21·0
7	2,112	3·15	10·3	21·8	10·1	21·3
6	2,622	3·30	8·6	22·5	7·7	20·2
5	1,711	3·20	11·4	19·5	10·1	17·3
4	1,525	3·45	13·5	20·6	11·6	17·7
*3	1,334	3·40	9·6	12·8	9·5	12·7
*2	611	3·15	15·1	9·2	15·2	9·3
*1	389	3·50	27·7	4·9	13·4	5·2

TABLE 3

Numerical Data for Solanum nigrum *Linn.*

Leaf no.	(A)	(P.R.)	(v.i.)	(Absolute v.i.) (A)×(v.i.) in thousands	(v.t.)	(Absolute v.t.) (A)×(v.t.) in thousands
8	885	4·6	13·0	11·5	13·3	11·8
7	793	4·5	14·9	11·8	14·2	11·3
6	460	4·8	21·9	10·1	22·0	10·1
*5	529	4·6	16·0	8·5	13·9	7·4
*4	276	4·2	34·9	9·6	27·9	7·7
*3	40	4·4	52·2	2·1	50·4	2·0

area of the lamina the vein-islet number and veinlet termination number increased and this increase is inversely proportional with the area of the lamina. For this reason (A)×(v.i.) and (A)×(v.t.) have been worked out and incorporated in the tables. These values are fairly constant in those leaves where the differentiation of the veins has been completed.

Because by multiplying the average number of vein-islets (or veinlet terminations) per square millimetre with the square millimetre area of the lamina a constant value is obtained, this may roughly represent the total number of vein-islets present in the lamina or the absolute number present.

TABLE 4

Numerical Data for Solanum ferox *Linn.*

Leaf no.	(A)	(P.R.)	(v.i.)	(Absolute v.i.) (A)×(v.i.) in thousands	(v.t.)	(Absolute v.t.) (A)×(v.t.) in thousands
6	10,284	5·6	7·1	73·0	3·2	32·9
5	11,340	5·5	6·1	69·2	3·3	37·4
4	9,870	5·4	7·5	74·0	4·1	40·5
3	5,308	5·0	13·9	73·8	6·7	35·6
*2	1,059	5·8	28·5	30·2	11·8	12·5

TABLE 5

Numerical Data for Withania somnifera *Dun.*

Leaf no.	(A)	(P.R.)	(v.i.)	(Absolute (v.i.) (A)×(v.i.) in thousands	(v.t.)	(Absolute v.t.) (A)×(v.t.) in thousands
8	1,400	6·7	22·2	31·1	15·9	22·3
7	1,396	6·2	22·0	30·7	16·6	23·2
6	995	5·8	27·6	27·5	21·7	21·6
*5	550	6·5	36·5	20·1	30·1	16·6
*4	128	6·9	48·8	6·2	45·2	5·8

This was found to be true not only in the leaves of a single branch of a particular plant, but also true when different plants of a particular species grown under different environments are considered. So these '*Absolute Numbers*' may be defined as follows.

ABSOLUTE VEIN-ISLET NUMBER: *When average vein-islet no. per sq. mm. of lamina of a mature leaf is multiplied by the sq. mm. area of the lamina, the Absolute Vein-islet Number of the leaf is obtained.*

ABSOLUTE VEINLET TERMINATION NUMBER: *When average veinlet termination no. per sq. mm. of lamina of a mature leaf is multiplied by the sq. mm. area of it, the Absolute Veinlet Termination Number of the leaf is obtained.*

The tables give the correlation between the area of the lamina and the vein concentrations in the five different species tested and the data are tabulated against each number of the leaf. The leaves in which the veins were found in different stages of formation have been marked with an asterisk (*).

RESULTS

From the tables 1–5 it is clear that the palisade ratio (P.R.) is remarkably consistent. But vein-islet numbers and veinlet termination numbers increase with the diminution of the areas of the laminae. This variation is found to be proportional, i.e. there is a negative correlation between the area of the leaves and their average vein-islet numbers or veinlet termination numbers. This is shown by the columns (A)×(v.i.) and (A)×(v.t.). These values approximately give the total number of these constants, termed here the 'Absolute Numbers'.

These correlations between the area of laminae and the vein-islet numbers or veinlet termination numbers were found constant not only in the individual plants for which illustrative data are given, but were also consistent when different individuals of the same species were taken into account. These correlations between different plants with their statistical analyses and the level of significance will be communicated in future.

SUMMARY

1. A method has been devised by which trichomes can be removed without injuring the epiderm of the leaves.

2. From each of the five plants analysed a branch containing leaves of various stages of growth, the palisade ratio, vein-islet number, and veinlet termination number as well as the area of the laminae were determined serially.

3. It was noted that while the palisade ratio remained more or less constant in a series, the vein-islet number and veinlet termination number increased with the decrease of the surface area of the lamina, and there is a negative correlation between these values.

4. By multiplying the average vein-islet number or veinlet termination number with the area of the lamina constant values are obtained. These constants are regarded as absolute numbers.

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Correlation of Tissues in Leaves

2. Absolute Stomatal Numbers

BY

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With one Figure in the Text

ABSTRACT

The average number of stomata per unit area of a leaf was found to be inversely proportional to the area of the lamina. The constant obtained by the multiplication of these values is termed as the Absolute Stomatal Number. This value is constant in those leaves where differentiation of the stomata has been completed. This has been shown with the developing leaves of five Solanaceous plants.

INTRODUCTION

THE number of stomata per unit area of the leaf surface has had little diagnostic value in describing leaves because of the high variation of this quantity from leaf to leaf and even at different portions of a single leaf. Yapp (1912), Timmerman (1927), Salisbury (1927), and some other workers found wide variations between the readings from a single leaf. They found highest frequency of stomata near the apex and lowest towards the base of the lamina. Also the margins had more stomata than the mid-rib. In the present work the same types of stomatal distribution in various species of the family Solanaceae were noted.

During an investigation on the quantitative microscopy of the leaves of the family Solanaceae, it was observed that there is a negative correlation between the area of the lamina and the vein concentrations (Gupta, 1960). At that time it was doubted whether there is any correlation between the area of the lamina and the average number of stomata per unit area.

MATERIALS

For the present investigation, developing leaves of five different plants of the family Solanaceae were selected. These plants are *Nicotiana tabacum* Linn., *N. plumbaginifolia* Viv., *Solanum nigrum* Linn., *S. ferox* Linn., and *Withania somnifera* Dun. All these plants were grown in this Institute in pots from the seeds of identified plants and those plants which were free from metabolic disorders were selected. The plants were pressed in a herbarium press and crumpling or folding of the leaves was prevented at the time of pressing. The leaves were then serially numbered, the numbering of the leaves beginning from the obviously protruding leaf bud at the end of the

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branch. Thus the first few leaves in each series are immature ones and the leaves of higher number are mature ones. The differences between the areas of the mature leaves and the immature ones were considerable.

METHODS

The general methods followed for the preparation of materials for the microscopic examinations were the same as described in Part I of this paper (Gupta, 1960). For the determination of vein-islet number and veinlet termination number there is a condition about the area required for counting, i.e. the counting should be made from 4 sq. mm. area (Wallis, 1953). But in the case of 'stomatal number' (number of stomata per sq. mm.), there is no such directive. For the determination of the minimum area required for counting stomata, an investigation was undertaken with the above-mentioned plants along with others and several sets of readings were taken from 0·1 to 1·0 sq. mm. area. It was observed from the data that when the number of stomata is counted from 0·4 sq. mm. area or more from lower epidermis of leaves, the readings become less variable and in these species five such readings give a consistent average. Commonly stomata are more uniformly distributed in the lower epidermis of the plants under observation and in the region midway between main vein and leaf margin. So in this paper only these stomata on the lower epidermis were estimated and the 'stomatal number' signifies the stomatal concentration of the lower epidermis of lamina only.

For critical examination of leaf epidermis, especially in the study of stomata, the need of separating the epidermal layer is often felt. In developing leaves and some types of thick leaves it may be difficult to distinguish the stomata from the surrounding cells in a whole-mount preparation. After several trials the following two methods of epidermis separation were adopted. Either fresh or dry leaves may be used. In some leaves, the epidermis can be peeled off from the fresh leaves but in most other cases one of the following two methods may be applied.

Nitric acid method. About 1 ml. of conc. HNO_3 (colourless) is taken in a test-tube and a little $KClO_3$ is added. Pieces of leaves (fresh or dry) are placed in the mixture which is then warmed in a low flame. Within seconds the leaves turn brown and then yellowish white with the evolution of bubbles. Before the leaves disintegrate the reaction is stopped by adding water. The materials are taken out and placed in a flat dish filled with water. If the leaves are thick, the epiderma can be picked out easily with needles under a dissecting microscope. But if the leaves are thin, the nitric-acid treated leaves are taken up in a test-tube half filled with water and agitated carefully and slowly till the epidermal layers are seen detached and floating in water. If the shaking is vigorous the leaves become fragmented. The right time for stopping the reaction is a matter of experience. If the reaction is carried out too long the whole leaf will be dissolved out, and if the reaction is stopped too early it will not be possible to detach the epidermis. For this purpose a few trials are needed to arrive at the optimum reaction period, the technique of warming, and of agitation &c.

Hydrogen peroxide method. The macerating action of hydrogen peroxide can also be utilized. This gives a slower process, but one easier to operate. For this purpose 2–3 ml. of hydrogen peroxide (20 vol. solution) is taken in a test-tube and a few drops of liquid ammonia are added. The leaf portions are added to this solution and the test-tube is plugged. The leaf portions may be kept in the solution by placing a small weight on them. The time required to complete the reaction may vary from 1 to 3 hours. At this stage the leaves become white and big bubbles are formed in the mesophyll. After a little washing the epidermis may be separated as in the above method.

The methods, however, did not give satisfactory results with leaves which had had the trichomes removed. In the case of woolly leaves, it is better to use the leaf portions as they are, removing the trichomes after separating the epidermal layers by either of the above methods. A slide is smeared with 'Mayer's Albumin Fixative' and flooded with water. Over this aqueous layer the detached epidermis are placed with their trichome-bearing surface on the top. Then the water is drained out and the epidermal layers are kept over the slide in stretched condition. The slide is then dried and the trichomes are brushed off with a small squirrel-hair brush. After the trichomes are completely removed a few drops of H_2O_2 are spread over the slide. When the tissue becomes loosened from the slide the epidermal layers can be detached with a fine jet of water.

The epidermis can be stained with aqueous gentian violet and can be mounted with 40 per cent. glycerine or with 'Glycerine Jelly', where, in place of phenol, sodium salicylate may be used as preservative. These are temporary preparations which remain serviceable for a couple of days. In some thin leaves there is no need to detach the epidermis for the observation and whole mounts of the leaves can be used. In such leaves (like *Solanum nigrum*) firstly the trichomes are removed by grinding (Gupta, 1960) and are cleared in chloral hydrate solution. Calcium oxalate crystals may be removed by acid treatment. After thorough washing they are spread over a slide with their lower epidermis on the top. With the help of a filter-paper excess moisture is removed and a drop or two of aqueous gentian violet is dropped on the centre of the leaf fragment to spread up to the margin of it, but prevented from spreading to the other surface. When the surface is deeply stained, the excess stain is washed away and is mounted with 40 per cent. glycerine with the stained surface on the top. In such preparation both the veins and the stomata of the lower epidermis can be estimated. After a couple of days the stain becomes diffused and so the analyses should be completed within this period.

In very young leaves it was found that the stomata were in the stage of formation and it is very difficult to distinguish between a stoma-mother cell and an ordinary epidermal cell. The leaves where the differentiation of stoma is going on have been marked with asterisk marks (*) in the tables and hollow points in the scatter diagrams. For the evaluation of the averages, five readings of stomatal number were taken from each leaf from the central portion of the lamina between mid-rib and margin.

OBSERVATIONS

In the following tables it may be observed that the stomatal number per sq. mm. of the lower epidermis is inversely proportional to the area of the lamina. For that reason when stomatal number (*S*) is multiplied by the area of the lamina (*A*), a constant value is obtained. This has been shown in the column (*A*)×(*S*), of the tables, as the average number of the stomata per sq. mm. of a leaf when multiplied by the square millimetre area of the lamina approximately gives the total number of the stomata of the surface, this may be termed the 'absolute stomatal number'.

TABLE 1

Numerical Data for Nicotiana tabacum *Linn.*

Leaf no.	Area of lamina in sq. mm. (*A*)	Stomatal no. (low) (*S*)	Absolute stomatal no. (*A*) × (*S*) in millions
18	34,080	92·6	3·16
17	32,890	101·8	3·35
16	32,670	118·0	3·86
15	30,100	120·6	3·63
14	24,390	148·8	3·63
13	20,240	185·4	3·75
12	19,130	189·4	3·62
11	17,470	216·2	3·78
10	15,050	219·8	3·13
*9	12,660	250·8	3·16
*8	8,241	300·6	2·48
*7	6,966	311·2	2·17
*6	3,322	352·6	1·17

TABLE 2

Numerical Data for Nicotiana plumbaginifolia *Viv.*

Leaf no.	(*A*)	(*S*)	Absolute stomatal no. (*A*) × (*S*) in millions
11	3,512	156·8	0·551
10	3,968	149·6	0·594
9	2,777	197·8	0·549
8	2,912	185·2	0·540
7	2,112	240·4	0·508
6	2,622	204·0	0·535
5	1,711	301·4	0·516
*4	1,525	306·2	0·467
*3	1,334	295·0	0·394
*2	611	328·6	0·201
*1	389	308·8	0·120

From the Tables 1 to 5 the total number of stomata (absolute stomatal number) per leaf was found to be more or less constant, except in leaves where

TABLE 3

Numerical Data for Solanum nigrum *Linn.*

Leaf no.	(A)	(S)	Absolute stomatal no. $(A) \times (S)$ in millions
8	885	349·2	0·309
7	793	372·0	0·295
6	480	516·6	0·238
*5	529	422·0	0·223
*4	276	403·4	0·111
*3	40	430·0	0·017

TABLE 4

Numerical Data for Solanum ferox *Linn.*

Leaf no.	(A)	(S)	Absolute stomatal no. $(A) \times (S)$ in millions
6	10,284	221·4	2·28
5	11,340	188·4	2·14
4	9,870	203·6	2·01
3	6,308	340·8	2·41
*2	1,059	530·2	0·35

TABLE 5

Numerical Data for Withania somnifera *Dun.*

Leaf no.	(A)	(S)	Absolute stomatal no. $(A) \times (S)$ in millions
8	1,400	204·4	0·286
7	1,396	220·4	0·308
6	995	266·6	0·265
*5	550	320·0	0·176
*4	128	346·8	0·044

all the stomata had not yet been produced. This has been shown in the scatter diagrams (Fig. 1 A to E), where the solid points represent the mature leaves and the hollow points represent those leaves where differentiation of the stomata has not yet been completed. From the tables as well as from the diagrams, it is evident that there is a striking negative correlation between the stomata and the area of the lamina. This relationship was found in the leaves of a single branch. When different individuals of the same species as the above plants grown in different environments were analysed, the same type of correlation was observed. The result of this experiment with statistical evaluation will form the subject-matter of another paper which will be communicated soon.

DISCUSSION

The high variation observed by Yapp (1912) and Timmerman (1927) in the stomatal frequency may be understood from the above findings. On the other hand, Salisbury (1927) found a positive correlation between the stomata and the ordinary epidermal cells—the 'Stomatal Index'. This quantity being

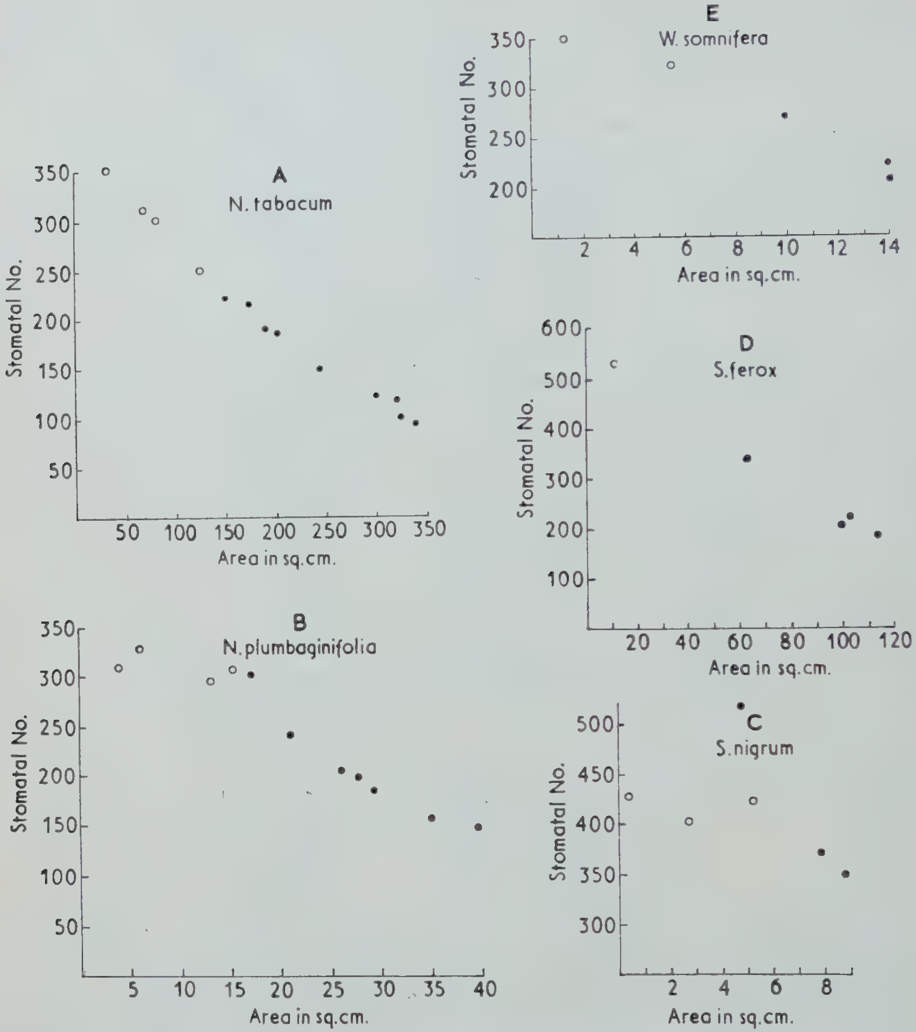

FIG. 1. The correlation between stomatal number and the area of the leaf lamina.

independent of area measurements, the effect of area on the stomatal concentration in these tissues has been neutralized. The effect, described by Maximov (1938) as the 'Rule of Zalensky', is found where the concentration of stomata and other tissues becomes high in the upper leaves of the plants, and may be due to the smallness of those leaves. Blackman and Rutter (1948) showed that the shading down to a level of 0·11 day-light causes little variation in the total leaf weight per plant; it nevertheless brings about a progressive increase in total leaf area to unit leaf weight. This can well be understood if it is assumed that, owing to shading, the leaf increases its surface area not by increasing the amount of tissues but by increasing the surface area of the cells and decreasing the thickness of the leaves.

Zörnig and Weiss (1925) showed that the ratio between the ordinary epidermal cell and the number of palisade cells beneath them is constant. Salisbury (1927) found that the proportion of stomata to ordinary epidermal cells is fairly constant. Now it has been shown that the vein concentration and the area of the lamina bear a negative correlation (Gupta, 1960) and also the stomatal concentration and the area of the lamina bear the same relationship in the leaves. All these observations may be correlated and it may be assumed that the relative proportions between different tissue systems within the lamina may be constant, and because the absolute numbers are fixed in a leaf, a definite amount of tissue is produced.

SUMMARY

1. Number of stomata per unit area of lamina is a very variable quantity. When this is counted from the central part of the lamina between mid-rib and margin and counted from 0·4 sq. mm. area, the variation is not so high. Lower epidermis of most leaves gives satisfactory results.

2. Two methods have been described by which the epidermis can be peeled from the leaves and the counting can be made with comparative ease.

3. The average stomatal number per unit area was found to have a negative correlation with the area of the lamina. The absolute stomatal number is obtained by multiplying the stomatal number by the area of the lamina.

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The Photoperiodic Reaction in Relation to Development in *Salvia splendens*

BY

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With two Plates and two Figures in the Text

ABSTRACT

Salvia splendens var. *Feu de la Saint Jean* was found to be a long-day plant. The maximal length of day in which the plants would not come into flower was, however, exceptionally low (7 hours). In all other aspects of its photoperiodic reaction the plant was typical of the long-day class. The plant showed a precocious sensitivity to the photoinduction of flowering. When induction was started at germination, flower induction was completed after giving 15 to 20 long days.

A comparison was made of the time required for the induction of flowering, as seen from the long-day to short-day transfer experiments, with the time required for the appearance of the first morphological signs of the onset of flowering, as seen in sections cut through the apical meristems. The morphological changes leading to the laying down of the first flower primordia took place a considerable time (15 days) after the completion of flower induction.

INTRODUCTION

IN the original photoperiodism experiments of Garner and Allard (1920) the authors raised their plants in a non-inductive day-length and, using such a culture as standard material, investigations were made into the photoperiodic treatment which was required to bring the plants into flower. This method was, and still is, used extensively by many of those undertaking experiments on photoperiodism. More recently, however, it has become apparent that the photoperiodic reaction of the plant differs throughout its life cycle (Harder and von Witsch, 1942; Harder and Springorum, 1947; Sironval, 1957; Petit, 1952; Mohr, 1959). The above authors all show that plants often possess a distinct photophase during which they are particularly sensitive to day-length as it affects flower induction. It is usually found that at no moment in its life cycle is the plant completely insensitive to the effect of day-length but instead manifests photoperiodic reactions other than flower initiation, e.g. changes in leaf form, pigment content, and production of epidermal hairs.

The object of the experiments to be described here was to determine the optimal photoperiodic treatment which would bring this variety of *Salvia splendens* into flower and to find how early in the development of the plant the flower induction could be made to take place. As well as investigating physiologically the moment of induction of flowering, a morphological

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examination was carried out to determine the relationship in time between the induction of flowering as seen physiologically and the onset of the first signs of the development of flower primordia in the apical meristem.

METHODS

Seeds of *Salvia splendens* var. Feu de la Saint Jean were obtained from Belot-Etienne (Liège) and sown in a standard soil mixture. On germination the seed boxes were transferred to the phytotron. (For description see Bouillenne and Bouillenne-Walrand, 1950.) Lighting was by means of Phytor tubes (Bouillenne and Fouarge, 1953) giving an intensity of 4,500 lux at a distance of 30 cm. from the centre of a battery of four lamps. Throughout the experiments the lamps were kept at a distance of 30 cm. from the uppermost leaves of the plants. The temperature was kept constant at 20° C. and the relative humidity was maintained at 80 per cent.

Once the seedlings had attained their first pair of true leaves they were transferred singly to 6-cm. pots. The plants were measured at regular intervals. In the transfer experiments, to determine when flower induction had taken place in the developing plant, the plants were transferred after the appearance of each node from the inductive to the non-inductive day-length (after the method of Sironval, 1957). After the completion of the transfers the effect of the induction treatment was measured as usual in the photoperiodic after-effect (Murneek and Whyte, 1948). Flower induction was considered to have taken place when the flowering buds could just be distinguished with the naked eye in 50 per cent. of the population of plants exposed to any one treatment.

Microscopic preparations were made from the apical buds from control plants in long and short days at the same moment as the transfers were made from one day length to another. In this way sections were obtained showing the development of the meristem at the appearance of each node from germination up to flowering.

RESULTS

The Effect of Day-Length on Flowering

Before undertaking the experiments on the induction of flowering with relation to development it was necessary to know the photoperiodic reaction of this variety of *Salvia splendens* in the conditions of culture obtained in the phytotron. Plants were grown in identically regulated growth rooms in day-lengths of 7, 8, 9, 11, 14, and 16 hours. At the same time a further group of plants was kept in continuous light (24 hours). Fifty plants were used in each test and the onset of flowering calculated as above. The results are illustrated in Fig. 1.

It is seen that with increasing day-length that the flowering of the plant was greatly accelerated. When the plants were kept in 8-hour days, 50 per cent. of the population were in flower after 110 days, whereas in day-lengths of 16 hours or more the plants were in flower after only 40 days. Prolonged observation of the plants in 7-hour days (6 months) failed to observe any plants in flower. However, it has since been observed that when the light intensity is doubled (9,000 lux) then a few of the plants in 7-hour days will

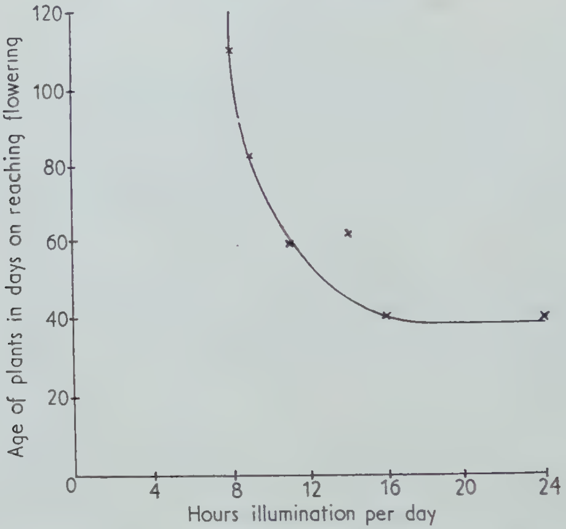

FIG. 1. Age of plants on flowering when grown in days of varying length

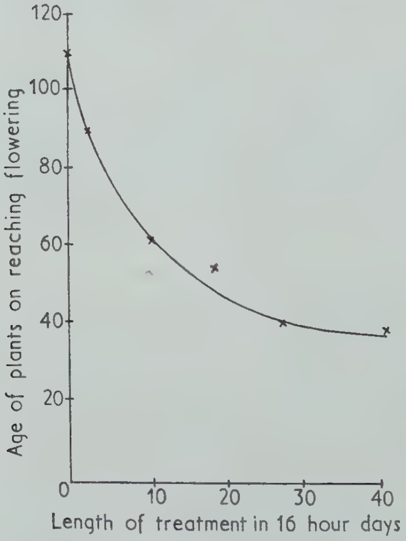

FIG. 2. Age of plants on flowering after being exposed from germination to varying lengths of induction treatment in 16-hour days.

form flowering buds, all of which eventually abort. (Gaspard, personal communication.) In this case the plants cannot be compared with the previous treatments as at no time was 50 per cent. of the population in flower and of those flowering buds which were formed none survived to produce functional flowers due to the premature abortion of the unopened buds.

From the form of Fig. 1 it is seen that the minimal day-length required for flowering not to take place is very low, in the region of 7 hours light per day. This, however, as with other long-day plants, is a definite lower limit under which flowering will not take place even if the intensity of illumination is increased.

Transfer Experiments

In order to determine at what moment in their development the plants were induced to flower the plants were transferred to a non-inductive from an inductive day-length at regular intervals (i.e. after the appearance of each node). From the results of the previous experiment on the effect of day-length on flowering it was decided for this purpose that the plants could be transferred to 8-hour days after an induction treatment in 16-hour days. It is true that the plants do not remain indefinitely vegetative in 8-hour days but as 110 days are required for the plants to come into flower in this day-length it was considered that this would leave a margin sufficient to observe the photoperiodic after effect of the treatment in 16-hour days.

In Fig. 2 is recorded the age of the plants on coming into flower in relation to the number of photoinductive cycles given after germination. It is seen that treatment with but a few 16-hour days greatly reduces the time necessary for the formation of flowers. Further treatment with photoinductive cycles in excess of 18 still reduces the time necessary for flowering but the gain in the time required to reach flowering is less for any given extra induction treatment than was obtained at an earlier stage. It appears, therefore, that the plant possesses no clear photophase. The photoperiodic induction of flowering is therefore not accomplished at any precise moment in the development of the plant but instead acts over an extended period starting at the moment of germination and continuing for a period of about 20 days.

Morphological Examination

While the plants for the transfer experiments were being raised in 16- and 8-hour days longitudinal sections were cut through the meristems of the control plants in both day-lengths at the same time as the transfers were made from the 16- to 8-hour days. Thus, sections were obtained after the appearance of each node until the plants were found to be in flower.

After examining a large number of sections it was found that only on reaching the fifth node after the plants had had at least thirty 16-hour days that the first morphological signs of the onset of flowering could be detected. Examples of typical meristems are shown in Plate 1 (1) and (2).

The rounding of the apical meristem of the fifth node is the first morphological sign that can be related to day-length and flowering. It thus appears that the induction of flowering as timed by the transfer experiments takes place without the simultaneous occurrence of any morphological change. It requires a further 15 days' growth and the appearance of two more nodes for the first signs to appear of the effect of flower induction on the development of the meristem.

Remarks on the Cessation of Flowering

It was noted in the above experiments that if the number of photoinductive cycles given was short of the amount required (i.e. 5 instead of 15 to 20) by the plants to come into flower with reasonable rapidity, then a large number of the flowering buds did not carry the floral development to its natural conclusion and produced functional flowers but instead the buds aborted without opening.

It would appear that in these cases that flower induction had partly taken place but was insufficient for the continued development of the buds. The plants then ceased to flower and reverted to the vegetative state. Struckmeyer (1941) found the same behaviour in another variety of *Salvia splendens* that bloomed in short days. To obtain successful flowering she found that induction had to be continued for a period longer than that which was necessary merely to initiate the formation of flowering buds.

This observation of the failure of complete flowering in minimal induction treatments raised the question as to whether it was necessary to continue the photoinduction treatment to ensure the continued production of flowers, or once the plant was fully induced and in full flower would flower production continue in the absence of further induction?

To obtain further information on this question of the reversibility of flowering, plants were raised as usual in pots in 16-hour days until the 72nd day after sowing. By this time the entire batch of plants (100) was well in flower. The plants were now divided at random into two groups. One group was replaced in the 16-hour days and the other was placed in 7-hour days. It was necessary to place the latter in 7-hour days, and not in 8-hour days as in the transfer experiments, as it had previously been shown that it was only the 7-hour day that was ineffective in inducing flowering with the light intensity used in these experiments (4,500 lux). Apart from these differences in lighting the plants were treated identically being kept in similarly conditioned chambers of the phytotron.

The plants in 16-hour days continued to bloom normally. By the time the plants were 4 months old the original flowering stalk from the terminal bud had been lost but the axillary buds had developed and produced flowering shoots. Plate 3 compares the state of development of the two sets of plants 47 days after they had been separated into the two groups. With the plants kept in 7-hour days the apical bud on the flowering stalk had aborted. The axillary buds then developed and did produce a few flowers before these buds, too, aborted. A further set of axillary buds then developed but these produced mainly leaves. In some branches a small bud with a few flower primordia was formed but this again soon withered and died. The abundance of leafy growth and the withered remains of the aborted flowering buds can be seen in the 7-hour day plants on the left of Plate 2. The 16-hour day plants continued to flower normally and both sets of plants remained healthy and made good growth.

It appears, therefore, that an induction treatment in 16-hour days does not endow the plants with the capacity to continue flowering in a day-length of 7 hours when they would normally remain vegetative.

Discussion

From the first experiments described it was seen that *Salvia splendens* var. Feu de la Saint Jean, is a long-day plant. With the intensity of light used the plant will probably remain in the vegetative condition indefinitely when grown in 7-hour days. Increasing the light intensity in 7-hour days cannot be said to induce flowering as the flower buds formed are never carried long enough on the plant to permit their development into functional flowers.

On transferring plants from long to short days at successive intervals it was found that the plants had only to remain 14 days in the long-day growth room for flower induction to have taken place. Transfers from long to short days, made before this length of induction treatment had been given, resulted in an increasing number of plants remaining vegetative and an increasing number of bud abortions among those plants that did produce flower buds.

However, no exact boundary existed between the time necessary for the successful induction of flowering and a treatment that would prove insufficient for the successful induction of flowering.

It was seen from the transfer experiments that it was between the appearance of the first and third nodes (i.e. between the first and fifteenth day) that flower induction took place in 16-hour days. However, the examination of the sections cut through the meristem show that it is only two nodes later (after a further 15 days' induction) at the fifth node that the first morphological signs appear of the induction of flowering. These results show in this variety of *Salvia splendens* that the condition of being induced to flower is not accompanied by any morphological change in the stem apex. It is only *two weeks later* just before the appearance of the flowering bud with the sixth vegetative node that the flower primordia are first laid down.

From the transfer experiments it was also seen that for flowering to be successful, i.e. for the buds to develop into functional flowers, a greater number of inductive cycles had to be given than was necessary for the formation of the first flower primordia. When this is considered together with the experiment on the cessation of flowering in plants in full bloom on transferring them to 7-hour days, it appears that for this plant the continued production of functional flowers requires a continuous induction treatment in long days. As the plants continue to grow healthily in the 7-hour days this cannot be dismissed as due solely to a period of photosynthesis insufficient for the plant's energy requirements.

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EXPLANATION OF PLATES 1 AND 2

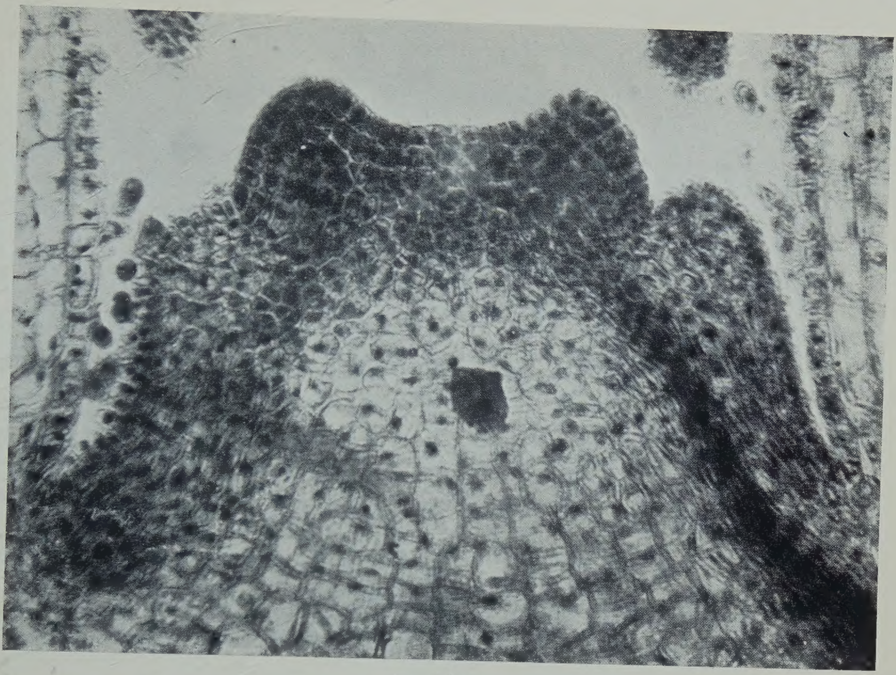
PLATE 1

FIG. 1. Longitudinal section of long-day bud from the first node showing typical vegetative appearance.

FIG. 2. Longitudinal section of long-day bud from the fifth node showing the first signs of the formation of flower primordia.

PLATE 2

Plants 117 days after sowing illustrating the cessation of flowering. On the left, plants transferred to 7-hour days after 72 days of 16 hours in length. On the right, control plants of the same age kept continuously in 16-hour days.



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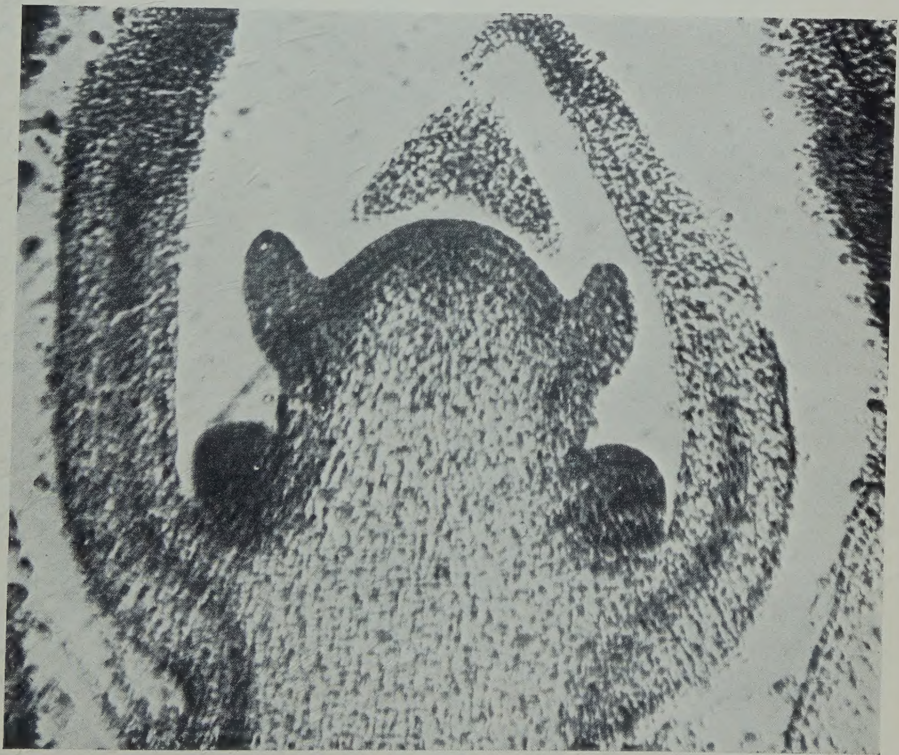

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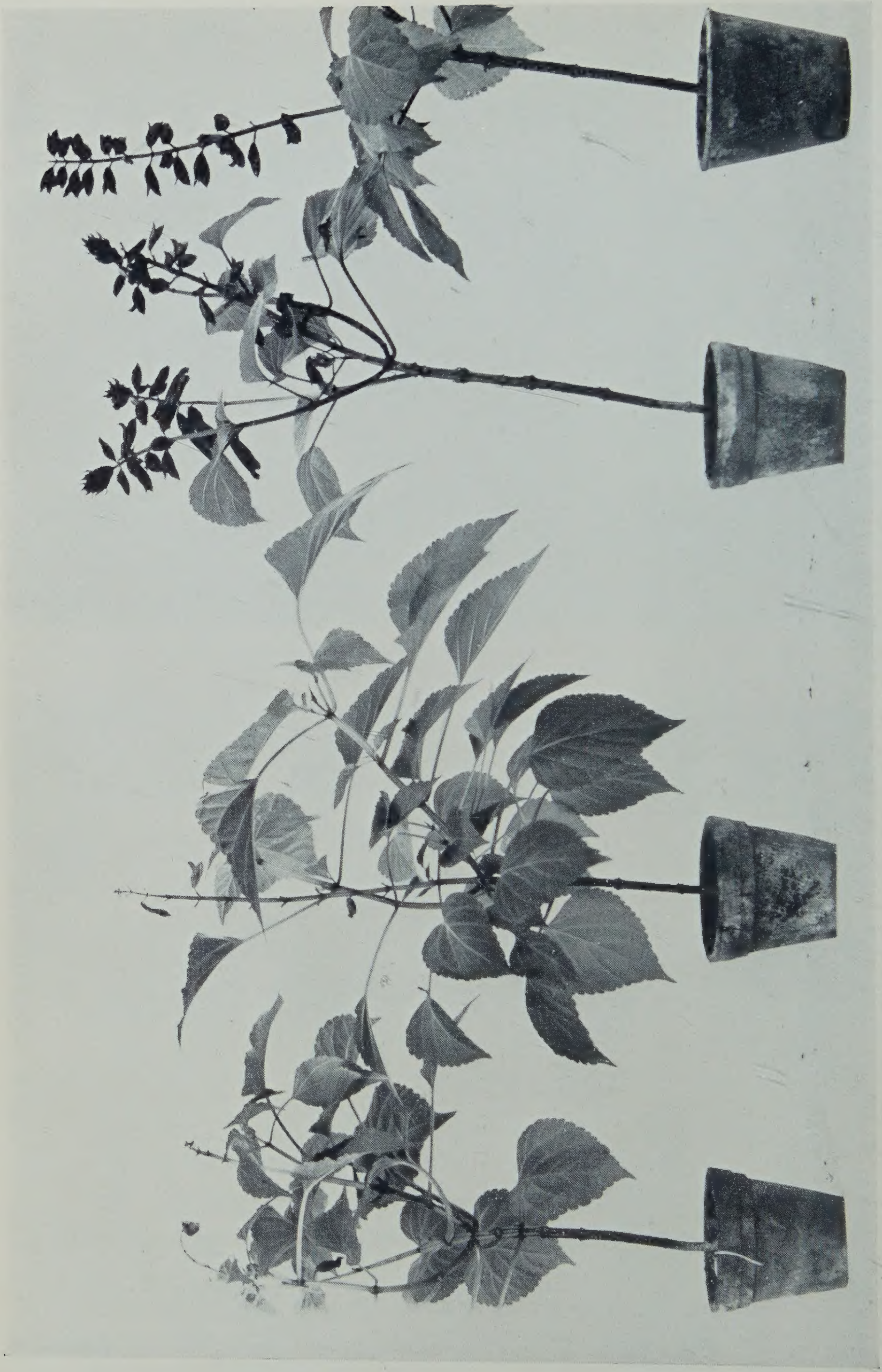

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